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Symposium on Energy Balance

Introduction

IN OCTOBER of 1958, the officers of The Upjohn Company invited us to collaborate with them in the organization of an international conference on obesity. Preliminary conversations with Dr. Robert Talley and other members of the Upjohn staff suggested to us the idea of attempting to recruit participants who could present many facets of the obesity problem as a part of the broad biologic concept of energy balance. We permitted our imaginations to run unrestrained and selected a panel of speakers and participants, who seemed to us to be best qualified to deal with the subjects to be discussed, or a sort of "ideal" or "fantasy" panel. To our considerable astonishment, what began as fantasy became reality for few of those originally invited to take part in this conference refused the invitation.

The design of the program called for a discussion of the regulation of food intake and the first session was devoted to this topic. The central structures involved in the integration of food intake behavior were referred to and there was an interesting discussion of the nature of the information that is received and interpreted as satiety in the central nervous system. In the second session, the subject of energy expenditure was introduced into the energy balance equation. The concept of the efficiency of the utilization of food was presented and a fascinating description of the rather special problems of energy balance of migratory birds was given.

The conversation then shifted to problems of intermediary metabolism, particularly the reactions involved in the processes of lipogenesis and cholesterologenesis. The malonyl coenzyme A pathway of fatty acid synthesis was described, and discussion of the regulation of cholesterol synthesis reduced the concept of balance to precise biochemical terms.

Following this discussion, the first of a number of papers on the general subject of the great energy bank of the body, the adipose tissue, and the conditions under which deposits and withdrawals of calories are made at the site of the aggregate depot organ was presented. The effects of hormones and of modifications in food intake pattern on fat deposits were of particular interest. The subject of withdrawals, the mobilization of free fatty acids from adipose tissue and its endocrine control, required one entire session of the conference, which was especially distinguished by the participation of Professor H. E. Wertheimer.

The final session was devoted to a discussion of genetically determined metabolic patterns that appear to predispose experimental animals and men to obesity. There is no doubt that such patterns exist in mice and there is a strong suspicion that similar ones occur in people; the difficulties of studying these problems in human populations were stressed.

The subject of the metabolic impact of long-continued obesity on the organism was one which could have been discussed in a symposium at least as lengthy as this one. Only two presentations were given in this area; one

on the susceptibility of obese mice to cancer and the other on certain hepatic and renal diseases that are commonly observed in hyperphagic rats.

The conference was marred only by the fact that Dr. William C. Stadie, who had been a sort of Unofficial Dean of metabolic physiology in this country, died a few weeks before the meeting. We are certain he would have enjoyed the discussion, and we missed his wise and gentle presence.

We are sure that the members of the conference join us in thanking the speakers, the discussers, and the chairmen of the sessions for adding so much to our education. We, the undersigned, were particularly pleased by the attendance and participation of our Chief, Dr. C. N. H. Long, to whom we are both enormously indebted for stimulating our interest in the subject of the conference twenty years ago.

To The Upjohn Company, which made the conference possible, and to Drs. R. W. Talley and C. J. O'Donovan, who did an outstanding job of organizing the meeting, we give our sincere thanks.

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Nervous Regulation of Food Intake

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CALORIC BALANCE depends upon the regulation of biologic energy exchange. Four important variables are concerned in energy balance: (1) food intake, (2) energy storage, (3) energy utilization through activity or work and (4) loss of energy in the production of heat. The caloric balance would thus be regulated by the various mechanisms which regulate the caloric intake in the form of food intake on the one hand and caloric loss on the other. In this communication the nervous mechanisms regulating food intake are discussed.

Cannon[1] in 1915 demonstrated rhythmic contractions of the stomach accompanying feelings of hunger, and on the basis of this, Carlson[2] built an entire theory of the regulation of appetite. Subsequent studies demonstrated, however, that this "peripheral" gastric mechanism was overemphasized as experimental observations showed that elimination of gastric contractions due to hunger or their central projection in a variety of ways did not alter the sensations of hunger.[3,4]

Meanwhile attention was focused on the central nervous system as a regulating mechanism for food intake. Hetherington and Ranson[5] demonstrated that obesity could be produced by lesions confined to medial hypothalamic regions, and Brobeck, Tepperman and Long[6] observed that such obesity was a result of hyperphagia. Hypothalamic hyperphagia was produced in several species of animals such as the rat, cat, dog and monkey.

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ROLE OF HYPOTHALAMUS

Anand and Brobeck[7] observed that hypothalamic injuries in rats could produce not only hyperphagia but also aphagia. Bilateral lesions in a restricted area of the lateral hypothalamus in the same rostrocaudal plane as the ventromedial nucleus, led to complete aphagia, and to death of the animal from starvation. On the other hand, lesions restricted to the medial hypothalamic regions of the ventromedial nuclei, and the regions in between these and the lateral hypothalamic areas, led to hyperphagia and obesity, provided that lateral hypothalamic centers were undamaged. It was suggested that the lateral hypothalamic area be designated the "feeding center" and the medial one the "satiety center."

Anand and his co-workers[8] extended these studies to include cats and monkeys and confirmed that the dual hypothalamic mechanisms regulated food intake. It was also demonstrated by Delgado and Anand,[9] and by Anand and Dua,[10] that electrical stimulation for one hour of the lateral hypothalamic areas in cats markedly increased their daily food intake on the days of stimulation, while stimulation of the medial hypothalamic areas for one hour produced only a slight decrease in their daily food intake on the days of stimulation. Complete aphagia never occurred. This finding was confirmed by Larsson[11] in goats.

These observations provided an anatomic basis for a possible central mechanism in the hypothalamus regulating food intake. It seemed that the lateral feeding mechanism was the basic urge, while the medial satiety mechanism acted by inhibiting the lateral mechanism for the following reasons: (1) Injury to the lateral regions invariably produces complete aphagia whether or not the medial regions are intact. (2) Injury to the medial regions produces hyperphagia only when the lateral region

is intact. (3) Stimulation of the lateral region markedly increases food intake, while stimulation of the medial region only slightly decreases it.

The nature of the nervous mechanism regulating feeding behavior would be expected to follow the same pattern as those serving other functions of the brain and spinal cord. These functions have been shown to have an important substructure of reflex actions with the brain stem, the diencephalon and the telencephalon leading either to their facilitation or inhibition. It may, therefore, be suggested that the regulation of feeding also is reflex in nature, with superimposed facilitation and inhibition from higher centers including the hypothalamus. It would appear that the lateral hypothalamic mechanism facilitates and the medial one inhibits the feeding reflexes.

The brain stem containing the nuclei of the cranial nerves is probably the level of the neuroaxis most directly concerned with the feeding reflexes, the motor and sensory components of which take part in normal feeding reflexes which aid in chewing, salivating, swallowing and in rejecting unacceptable objects. This was indicated as early as 1916 by Miller and Sherrington.[12]

Brobeck[13] has proposed two different tentative classifications of feeding reflexes. One is based on the nature of the stimulus inducing the reflex which may be tactile, gustatory; auditory, or visual, by making the animal aware of food, or enteroceptive from the gastrointestinal tract. The second is based on the sequence of behavior in normal feeding, including reflexes of attention, approach, examination, incorporation and rejection.

CEREBRAL INFLUENCES

Experimental work has also suggested cerebral influence over normal feeding responses. Pribram and Bagshaw[14] reported increased food intake after surgical lesions involving temporal polar-amygdaloid formations in dogs. Anand et al.[8] had observed that bilateral destruction of hypothalamic feeding centers in some monkeys produced a response somewhat different from that seen in cats and rats with similar lesions. Such monkeys would not eat the food available in the cage although they would swallow it when it was put directly into their mouths. Aphagic cats and rats with similar lesions would always reject the food even when it was put into their mouths. It was presumed that this difference in the monkeys' behavior was due to higher encephalization, suggesting that, at least in primates, control over food intake was also mediated through higher cerebral centers.

Anand, Dua and Chhina[15] further demonstrated that bilateral lesions in certain portions of the cerebral cortex or of the limbic system altered the intake of food in monkeys and cats. For example, lesions of the frontal lobe, including or restricted to the posterior orbital cortex, led to a decrease in food intake, but those involving only the frontal tips and sparing the posterior orbital cortex led to an increase. Extensive lesions of temporal lobe also led to an increase in the intake of food; lesions in the amygdaloid and periamygdaloid regions of the temporal lobe produced temporary aphagia; but lesions restricted to anterior cingulate gyri had no effect. Those changes observed in intake of food were more noticeable in monkeys than in cats, were never so pronounced as those associated with lesions of the hypothalamus, and also tended to disappear after some weeks. Therefore, it was concluded that the limbic structures in the frontal and temporal lobes modified the intake of food through a discriminating mechanism (appetite), while the primitive urge (hunger) originated at the hypothalamic levels. From experiments with rats, Bruce and Kennedy[16] had also postulated a similar hypothesis. The cerebral influences are possibly mediated through the hypothalamus modifying the effects of hypothalamic centers.

Stimulation of the various limbic structures did not produce any change in the daily intake of food. However, such stimulation produced responses of chewing, licking, sniffing, salivation and repetitive opening and closing of the mouth with protrusion of the tongue; these have been grouped under the heading of "eating" automatisms.[17] Eating responses were also produced during stimulation of the lateral hypothalamic feeding centers,[9,10] but these

were accompanied by an increase in the intake of food.

We[18] have also produced bilateral lesions in some neocortical regions. We observed that there was a slight change in food intake for a short time only after lateral frontal lesions had been produced; while lesions in the parietal, occipital and temporal neocortical regions did not produce any change in food intake.

Enough evidence is thus available to classify the central nervous mechanisms regulating food intake in a manner similar to that employed in the classification of regulatory mechanisms for other autonomic and visceral activities, such as the regulation of blood pressure, pulmonary ventilation and body temperature. Feeding behavior is probably regulated by certain reflex mechanisms mediated from the spinal cord and brain stem levels, which are definitely facilitated and inhibited from the hypothalamic regions and further regulated from the higher cerebral limbic and possibly neocortical regions. In common with the other visceral reflexes, the regulation from the limbic levels is more pronounced than from the neocortical levels.

MECHANISM OF NERVOUS REGULATION

Since adult men and animals can maintain their body weights, and since growing organisms continue to grow at well defined rates, food intake must be adapted to caloric needs. Gasnier and André Mayer[19] demonstrated that animals varied their food intake in a way which indicated that two regulations were at work. One, which is more important than the other, adjusts the calories eaten to the calories spent from day to day; and the other, working more slowly, corrects over a period of time whatever error the rapid mechanism could have made.

A simplified explanation for these adjustments would be that when food is eaten by a normal animal, certain changes occur within the body which either directly or indirectly affected the hypothalamic centers and possibly also the higher cerebral centers, thereby changing the feeding reflexes. These changes stimulate the activity of the medial or inhibiting hypothalamic mechanisms and suppress the lateral facilitating mechanisms, thus producing satiety. On the other hand, when the food eaten is disposed of through conversion to heat, work or some form of stored energy, the changes produced by the feeding tend to disappear; consequently, activation of the satiety mechanism is removed and the lateral facilitating mechanism becomes more active leading to a state of hunger.

Various suggestions have been made regarding the nature of the change or changes, produced as a result of feeding, which influence the regulating system. The factors suggested by various workers are the following: (1) the specific dynamic action of food, increasing the heat stress of the body as a whole (the thermostatic hypothesis of Strominger and Brobeck[20]); (2) the availability and utilization of glucose from body fluids (the glucostatic hypothesis of Mayer[21]); (3) the concentration of certain metabolites, as yet unspecified (the lipostatic hypothesis of Kennedy[22]); (4) concentration of serum amino acids[23] and (5) sensations from the digestive tract associated with eating, swallowing and the presence of food in the stomach and in the intestine.[24,25] The ingestion of a single meal is accompanied by a number of changes in the animal body and more than one such change could act as a signal to the regulatory mechanism. On the basis of existing evidence, it would seem unwise to incriminate a single specific factor. A multiple factor theory of regulation appears to be most reasonable.

The two hypotheses of regulation of food intake, which today compete for emphasis, are the "thermostatic" and the "chemostatic" (especially the glucostatic) regulation hypotheses.

THERMOSTATIC REGULATION OF FOOD INTAKE

Brobeck wrote, "animals eat to keep warm, and stop eating to prevent hyperthermia."[20a] He and his colleagues[20,26] concluded that the day-to-day regulation of food intake is not in terms of a definite quantity of energy (a quantity equal to the total expenditure of energy); instead it is the specific dynamic action of the ration which determines the amount of food eaten. Rats placed on a high fat diet often ingested three times their normal caloric

intake on the first day following the change in diet. Another point in favor of the thermostatic regulation is that there is no direct evidence for a specific sensitivity of the hypothalamic neurons, except to temperature change.[13]

CHEMOSTATIC REGULATION OF FOOD INTAKE

For short-term regulation of energy intake, Mayer[21,27,28] proposed the glucostatic theory, which postulates that somewhere, possibly in the hypothalamus, there are glucoreceptors sensitive to blood glucose in the measure that they can utilize it. He reasoned that during the interval between meals the content of fats and proteins within the body, which are proportionately enormous, would decrease insignificantly; while the stores of carbohydrates, which are limited, would decrease proportionately more. Glucose is the essential fuel of the central nervous system.

It seemed reasonable to postulate, therefore, that hypothalamic centers may be glucoreceptors. Mayer and Bates[29] showed that in normal and diabetic animals and in animals subjected to various hormonal treatments, decreased availability or utilization of glucose correlated well with increased food intake. A generally reliable representation of the utilization of glucose can be obtained from the arteriovenous differences of glucose (Δ-glucose) rather than from absolute levels of blood glucose.[30] It was demonstrated that hunger is a state in which the Δ-glucose tends toward zero while in the state of satiety there is an appreciable Δ-glucose. Stunkard and Wolff[31] found that small Δ-glucose coincided generally with gastric contractions due to hunger and subjective feelings of hunger in human beings, while large Δ-glucose accompanied satiety and disappearance of contractions of the stomach.

Mayer and Marshall[32] also demonstrated that injection of gold thioglucose in mice produced overeating and obesity by causing selective destruction in the medial satiety centers of the hypothalamus, while other gold thio-compounds did not produce any hypothalamic lesions or obesity. It was suggested that the affinity of the glucoreceptors in the ventromedial nuclei of the hypothalamus for the glucose moiety of the compound causes the glucoreceptors to accumulate proportionately more gold than other regions, sufficient to damage these particular neurons. Perry and Liebelt[33] have demonstrated that gold thioglucose, in addition to producing lesions in the ventromedial nuclei and obesity, also produces extrahypothalamic lesions. All the loci where these lesions are produced have in common a proximity to areas with increased permeability of the blood-brain barrier.

Anand, Dua and Singh have also studied this problem using a different method. Depth electrodes were implanted in the lateral feeding and the medial satiety hypothalamic centers of monkeys and cats and the activity of these regions were recorded electroencephalographically. After taking the normal recordings, blood glucose levels were changed either by intravenous infusion of glucose saline or by intravenous injection of insulin. Control electrodes were also implanted in the other hypothalamic and cortical regions. It was observed that with the production of hyperglycemia the activity of the satiety center was increased while that of the feeding center was slightly decreased. With hypoglycemia, the activity of the satiety center was slowed down while that of the feeding center was increased. These changes were observed both in monkeys and cats. Changes in blood glucose levels did not alter the recorded activity from the other hypothalamic and cortical regions. Intravenous transfusion of protein hydrolysate did not alter the activity of either the feeding or the satiety centers. Larger doses produced a generalized inhibition of activity as a result of protein shock. Intravenous infusion of fat emulsion (Lipomul®) also did not change the activity of hypothalamic centers. Therefore, our studies support the hypothesis that the hypothalamic centers are sensitive to changes in blood glucose, rather than changes in blood protein or fat.

Forssberg and Larsson[34] demonstrated that in hungry rats the uptake of P^{32} was greater in the regions of the hypothalamus, which have the feeding mechanisms, than in other regions. Their studies of the uptake of glucose containing C^{14} were inconclusive. Studies of the uptake of glucose by the feeding and satiety

regions of the hypothalamus in fed and starving monkeys have been made by Anand, Talwar, Dua and Mhatre. In the first series of experiments, glucose containing C^{14} was injected into the carotid arteries of both fed and starving monkeys. The monkeys were beheaded immediately after the injection was given and the brains were frozen in liquid air. Pieces from the satiety and the feeding regions and from two other adjacent hypothalamic regions were studied then for radioactivity. No significant activity was detected in any region.

In other experiments, uptake of glucose and oxygen by various hypothalamic regions in fed and starving monkeys has been investigated with the Warburg technic. Preliminary observations suggest that in the fed animals there is a relative increase in the uptake of oxygen and glucose per unit of nucleic acid activity by the satiety region, as compared with that of the feeding center. In the starved animal, the uptake of oxygen and glucose by the satiety region is less than that of the feeding region. The arteriovenous glucose difference was low in starved animals and high in the ones which had been fed. These results indicate an increase in activity of the satiety centers during fed states, which is accompanied by an increase in the uptake of glucose. They also suggest that the satiety region of the hypothalamus contains the glucoreceptor mechanism. They also support the original hypothesis of Anand and Brobeck,[7] that the basic urge to eat is located in the lateral hypothalamic regions; while the medial regions are activated as a result of changes in the levels of the blood sugar produced by food intake, which subsequently produces satiety and abolition of further eating by inhibiting the lateral mechanisms. The electroencephalographic recordings from feeding and satiety centers under conditions of hyperglycemia and hypoglycemia (mentioned previously) lend further support to this hypothesis, as the changes in the activity of satiety centers are more pronounced than changes in the activity of feeding centers.

AFFERENTS FROM THE STOMACH

Experiments using the evoked potential technic are being conducted to determine whether or not the gastric afferent impulses, traveling centrally through the vagus, project to the hypothalamic centers. Preliminary studies have shown a projection into the medial hypothalamus just anterior to the satiety center. Ballooning of the stomach, *in situ*, also evokes potentials in the medial hypothalamic regions, which correlate well with increasing intragastric pressures.

CORRELATION OF WATER INTAKE WITH FOOD INTAKE

Strominger[35] had noted that, within limits, the higher the water concentration of the diet, the greater the food intake; animals given no water ate little or no dry food and those given no dry food drank little or no water. The regulation of food intake appears to be correlated with the regulation of water exchange. It has been suggested by various authors that, if these two are so intimately correlated, after hypothalamic lesions have been produced the changes in food intake may be the indirect result of the changes in water intake which may be the primary function regulated from the hypothalamic levels. Anderson and McCann described a hypothalamic "drinking area" which produces polydipsia when electrically stimulated.[36] Similar results were obtained by microinjection of hypertonic saline into the same regions. Montemurro and Stevenson[37] demonstrated that the hypothalamic area regulating water intake was situated in the same region as the feeding center. Studies of Anand and Dua[38] have demonstrated that, after hypothalamic lesions have been produced, rats fail to show the correlation of water with food intake observed in normal animals. Lesions in the lateral hypothalamic feeding center resulted in complete adipsia in addition to complete aphagia. Lesions near this region (1 mm. medial or anterior) produced hypodipsia, irrespective of increase in food intake. Lesions farther away from this region did not significantly change the water intake despite changes in food intake. It can be concluded, therefore, that the hypothalamic regions controlling water and food intake, although present in adjacent regions, act separately and independently. Morrison and Mayer[39] have made similar observations. It may be noted here

that lesions in the limbic structures of rats do not change their water intake.

SUMMARY

On the basis of studies presented herein, it is possible to say that the mechanisms of the nervous regulation of food intake can be categorized with those governing the nervous regulation of other visceral activities. Experimental evidence has been furnished suggesting certain modes of activation of the higher nervous mechanisms. More knowledge is required before a complete picture of the nervous regulation of food intake is finally revealed.

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DISCUSSION

Dr. C. N. H. Long (*New Haven, Connecticut*): Dr. Anand, why is the diabetic animal hungry?

Dr. Anand: I do not think I can give a straight answer to this. If one were to hypothesize why a diabetic animal is hungry, an explanation might be that although the blood sugar is high, there is less sugar available within the cells of the satiety center.

Dr. Rachmiel Levine (*Chicago, Illinois*): There is no evidence that neurons need insulin for consumption of glucose. Perhaps these neurons are different, but it would first have to be demonstrated.

Dr. Jean Mayer (*Boston, Massachusetts*): I do not want to anticipate on my paper, however, there is considerable and cumulating evidence that those neurons are different.

Dr. Henry D. Janowitz (*New York, New York*): I would like to ask Dr. Anand, who has shown us that the satiety center is activated by distention of the stomach, whether he has picked up any spontaneous activity of the feeding center during spontaneous or hunger contractions.

Dr. Anand: Ordinarily we have not seen any marked changes in the activity, either from the satiety or the feeding areas, but when we inflate the stomach with a balloon, we find that there is increased firing from the satiety area only and not from the feeding area.

Metabolic Effects of Glucagon in the Wistar Rat

JAMES M. SALTER, PH.D.*

SHORTLY after the discovery of insulin by Banting and Best, it was noted by Murlin and Kimball[1] in 1923 that the hypoglycemia produced by extracts of pancreas was preceded by a transient but significant elevation in the blood sugar concentration. Murlin postulated that this initial hyperglycemia was due to a second hormone in the pancreas which he named glucagon. Despite this early recognition glucagon awakened the interest of few investigators and knowledge of its biological properties progressed slowly. Through the efforts of Burger and Kramer[2] and Sutherland et al.,[3,4] glucagon was partially purified and its was shown that, like epinephrine, it acted directly on liver tissue (but not on muscle) to stimulate the breakdown of glycogen. Because of this effect, glucagon was called the pancreatic "hyperglycemic glycogenolytic factor," a term used rarely today.

For many years the glycogenolytic effect appeared to be the only significant action of glucagon. However, its purification and eventual crystallization by Staub and co-workers[5] at the Lilly Research Laboratories in 1953 greatly facilitated experimental studies. It has been shown in many laboratories that under experimental conditions glucagon produces an array of metabolic changes. In addition to stimulating hepatic glycogenolysis, glucagon increases amino acid catabolism, urea synthesis,[6,7] ketone body production[8,9] and electrolyte excretion.[10]

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The primary purpose of our investigation was to obtain information on the effects these metabolic alterations would have on growth and body composition. The unexpected results of the study indicate that glucagon can significantly influence energy balance in the rat.

MATERIALS AND METHODS

Male Wistar rats, weighing approximately 142 gm., were used. The animals were housed in individual metabolism cages in a room maintained at a constant temperature of 28 ± 1°c. Their diet consisted of the following ingredients: casein, 50 gm.; Drackett soya protein, 130 gm.; cystine, 1 gm.; starch, 150 gm.; sucrose, 496 gm.; lard, 100 gm.; Cellu flour, 20 gm.; choline chloride, 3 gm.; B-complex vitamin mixture, 10 gm.[11]; corn oil solution of vitamins A, D and E,[11] 10 gm.; and salt mixture,[11] 10 gm. (total weight, 1 kg.).

During the experimental period the food intake and weight of each animal was determined daily. The urine and feces of each rat were collected separately every twenty-four hours and their nitrogen contents determined by the Kjeldahl technic. The sodium concentrations of urine were determined by flame photometry. Blood glucose and cholesterol concentrations were estimated according to the methods of Somogyi[12] and Zlatkis,[13] respectively. Crystalline glucagon (Lilly) was suspended in 0.9 per cent saline at a concentration of 200 μg. per ml. and injected subcutaneously.

Organs were removed at necropsy, weighed, and after a small piece was taken for histologic examination, returned to the carcass. The analyses of the carcasses for total body water, fat and protein were performed by technics previously described.[14] The amount of insulin

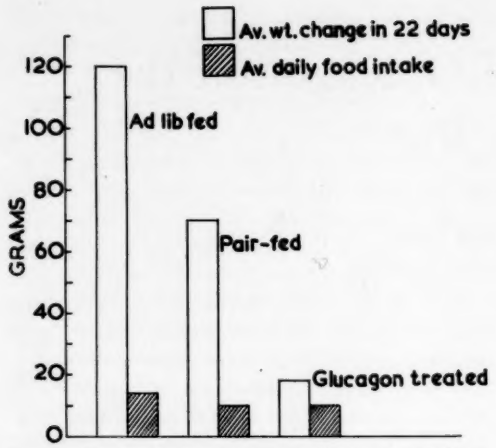

FIG. 1. Body weight changes and food intake of glucagon-treated rats and control animals.

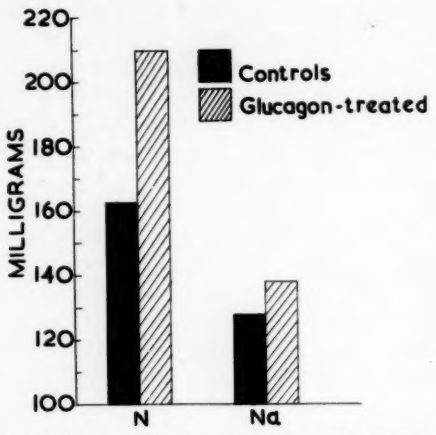

FIG. 2. Average amount of nitrogen and sodium excreted daily in the urine of glucagon-treated rats and their pair-fed controls.

extracted from the pancreas was determined by the method of Scott and Fisher,[15] and concentrations of liver glycogen by the procedure of Good et al.[16]

EXPERIMENTAL PROCEDURE AND RESULTS

Forty rats were divided into three groups. The fifteen animals in group 1 were each given subcutaneous injections at roughly eight hour intervals of 40 μg. of glucagon suspended in saline. The fifteen rats in group 2 were used as controls. They were given injections of saline, and limited to the same amount of

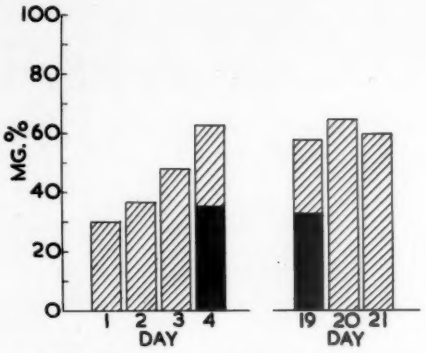

FIG. 3. The diagonally lined bars show the average change in the blood sugar concentration thirty minutes after the morning injection of 40 μg. of glucagon in rats treated for twenty-two days with this substance. The black portions of the bars show the change in the rats treated with a single injection of glucagon on the fourth and the nineteenth day.

food consumed by the treated rats in the first group. Group 3 consisted of ten rats which were used to show normal standards. They were given injections of saline and ate *ad libitum*. All animals were sacrificed on the twenty-third day. The results are presented in Tables I to III and in Figures 1, 2 and 3.

Body Weights and Food Intake. The glucagon-treated rats gained only 18 gm. during the experimental period while the rats in the control group, which were limited to identical amounts of food (an average of 10 gm. daily), gained 71 gm. (Fig. 1).

The second group of rats which ate *ad libitum* consumed an average of 4 gm. more food than the pair-fed control animals and gained 49 gm. more weight (a total of 120 gm.).

The data presented in Table I show that the glucagon-treated rats gained less weight than the untreated pair-fed animals because they retained less water and synthesized less fat and protein.

With the exception of the liver, which was abnormally heavy, the visceral and endocrine organs of the glucagon-treated animals were lighter than those of the pair-fed control animals and reflect the general inhibitory effect of glucagon on growth (Table II).

Urine. Although large doses of glucagon produce symptoms of diabetes in many species,[7,8,17] neither glucosuria nor ketonuria

TABLE I
Carcass Composition

Treatment	Animals in Group (no.)	Average Body Weight (gm.)	Average Weight Change	Average Composition (%)				Average Weight (gm.*)		
				Water	Fat	Protein	Undetermined	Water	Fat	Protein
Glucagon	15	164	18	65.30	12.37	18.26	4.07	107.40 ± 1.52	20.60 ± 0.28	30.10 ± 0.60
Control animals pair-fed	15	212	71	67.20	11.40	18.28	3.22	142.44 ± 1.67	24.13 ± 0.25	38.76 ± 0.53
Control animals, fed *ad libitum*	10	261	120	64.77	14.28	17.63	3.32	169.32 ± 1.92	37.66 ± 0.31	46.08 ± 0.56

* ± Standard error of the mean.

TABLE II
Organ Weights*

Treatment	Rats in Group (no.)	Average Body Weight (gm.)	Liver (gm.)	Kidneys (gm.)	Heart (gm.)	Adrenals (mg.)	Thyroid (mg.)	Thymus (mg.)	Testes (gm.)	Pancreas (mg.)
Glucagon	15	164	9.84 ± 0.25	1.30 ± 0.010	0.56 ± 0.015	25.1 ± 0.91	12.1 ± 0.83	160 ± 12.3	2.45 ± 0.11	525 ± 22.0
Control animals pair-fed	15	212	6.75 ± 0.17	1.59 ± 0.015	0.65 ± 0.016	30.1 ± 0.80	14.8 ± 0.67	406 ± 13.6	2.60 ± 0.08	694 ± 17.3
Control animals fed *ad libitum*	10	261	11.46 ± 0.39	1.94 ± 0.019	0.81 ± 0.016	35.0 ± 0.88	20.4 ± 0.95	494 ± 18.7	2.93 ± 0.13	839 ± 26.4

* ± Standard error of the mean.

TABLE III

Treatment	Average Insulin in Pancreas (units/gm.)	Average Liver Glycogen		Average Blood Cholesterol (mg./100 ml.)	Average Blood Sugar (mg./100 ml.)	Average Fecal Nitrogen/Day (mg.)
		mg.*	%			
Glucagon	1.34	492 ± 42	5.0	78.3 ± 3.3	86 ± 3.9	14.0
Control animals, pair-fed	1.82	209 ± 33	3.1	113.0 ± 3.7	91 ± 4.2	15.5
Control animals, fed *ad libitum*	1.68	504 ± 53	4.4	114.0 ± 4.1	104 ± 3.1	...

* ± Standard error of the mean.

occurred during these experiments. The treated rats consistently excreted about 60 mg. more urine nitrogen daily than the pair-fed control animals (Fig. 2). The nitrogen content of the feces was unaffected (Table III).

The administration of glucagon temporarily increased the sodium in the urine. During the first two days, the rats in the control groups excreted an average of 142 ± 6* mg. sodium daily, while the treated animals excreted 176 ± 9 mg. sodium. The excretion of this metabolite fell to values that averaged slightly higher than those of the pair-fed control animals although the difference was not significant.

* Standard error of the mean.

Blood. Estimation made on blood sugar at 8.30 A.M. (before the morning injection of glucagon) on days 1, 2, 3, 4, 19, 20 and 21 failed to reveal any difference between the blood glucose concentration of the pair-fed control animals and the glucagon-treated rats (Table III). However, the hyperglycemic response in the glucagon-treated rats increased during the first four days of treatment and then remained constant through the remainder of the experimental period (Fig. 3).

Blood cholesterol levels were determined on the last day only, and showed that the concentration of cholesterol was significantly less than that found in the control animals (Table III).

The concentration of glycogen in the livers of the glucagon-treated rats was more than twice that found in the control groups (Table III). Estimation of the insulin extractable from the pancreas indicates that the concentration of this hormone was significantly reduced by the administration of glucagon.

COMMENTS

It has been reported that the administration of glucagon will produce temporary, but severe diabetes in force-fed rats, and in rabbits and human subjects on normal dietary regimens.[7,8,17] Diabetes did not develop in the experiments described here because (1) the dose of glucagon (120 μg. daily) was only one-tenth of that used in earlier investigations and (2) the rats ate approximately 30 per cent less food than they did prior to treatment. The reduction of insulin content in the pancreas and the elevation in glycogen concentration of liver suggests, however, that carbohydrate metabolism was altered appreciably.

It has been reported by Costa et al.[18] and by Root[19] that although an injection of glucagon causes an immediate decrease of liver glycogen this is followed by a rapid increase to levels greater than normal. The high level of liver glycogen found in our experimental animals was in accord with these observations, and probably explains the potentiation of the hyperglycemic effect of the glucagon that occurred during the early phases of this investigation.

The most striking change was the effect of growth suppression by glucagon. The treated rats gained 70 per cent less weight than the control rats while consuming identical amounts of food. Although the increase in amino acid catabolism and nitrogen excretion induced by glucagon[7] explains the suppression of protein synthesis, it would be expected that the accompanying increase in gluconeogenesis would in turn potentiate lipogenesis. This did not occur; fat synthesis was definitely reduced.

This observation, i.e., that the potential caloric value of the carcasses of the treated rats was much less than that of the control rats despite the equality of their caloric intakes, suggested that the production of heat in the rats treated with glucagon may have been elevated. This led to an investigation of the effects of glucagon on oxygen consumption. The results of the latter studies are presented in another paper.[20]

SUMMARY

Male Wistar rats, weighing 145 gm., were given injections of 40 μg. of glucagon every eight hours for twenty-two days. They gained 75 per cent less weight, synthesized much less protein and fat and retained less water than control animals restricted to the same amounts of food. The glycogen content of the livers from the treated animals was more than twice that found in the control animals. In glucagon-treated rats, the hyperglycemic response increased during the early phases of the investigation, but glucosuria and ketonuria never occurred. The treatment produced a transient increase in the urinary excretion of sodium and a sustained increase in the urinary excretion of nitrogen. Fecal nitrogen was not affected. It was also observed that glucagon reduced the insulin content of the pancreas.

The fact that glucagon-treated rats synthesize less fat and protein than untreated control animals, consuming identical amounts of food, indicates that this hormone exerts a significant influence on caloric balance.

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The Effect of Glucagon on the Metabolic Rate of Rats

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DATA presented in a previous report showed that rats treated with small amounts of glucagon gained less weight and contained less protein, fat and water than pair-fed control rats.[1] Since the caloric intake of the two groups was identical, although the caloric value of the carcasses of the glucagon-treated animals was much lower than that of the control rats, the effect of glucagon on the metabolic rate was studied. The results of the investigation indicate that glucagon, under experimental conditions increases heat production thus altering caloric balance.

MATERIALS AND METHODS

Male Wistar rats weighing 200 to 250 gm. were fed Purina chow *ad libitum* and kept in a room maintained at a temperature of 28° ± 1°c. for a period of at least ten days before being subjected to experimental procedures. The thyroidectomized animals were used after a postoperative period of one month. Cessation of weight gain and subnormal oxygen consumption were accepted as evidence that the thyroid gland had been completely removed. The adrenalectomized animals were given 1 per cent saline to drink while those treated with cortisone were given a choice of saline or tap water. Upon completion of the investigation the adrenalectomized rats were sacrificed and then autopsied. The sites of the suprarenal glands were carefully examined through a dissecting microscope. No evidence of adrenal remnants were found.

Adrenal demedullation was performed by the enucleation technic of Evans.[2] The animals were given a 1 per cent sodium chloride solution to drink for seven days postoperatively. Three weeks were allowed for regeneration of the adrenal cortices. At autopsy, histologic examination of the glands showed no evidence of chromaffin tissue.

Crystalline glucagon§ was administered subcutaneously either as a suspension in neutral saline or as a solution in saline at a pH of 9 to 10. Injections of crystalline serum albumin‖ in neutral saline or in solution at a pH of 9 to 10 were administered to control animals as required. Adrenalin (epinephrine)¶ and cortisone** were administered intramuscularly.

All rats were fasted four hours before the experiments. Their metabolic rate was measured in terms of the oxygen consumption of each animal. For each experiment four treated and four control animals were used. The oxygen consumption was determined according to the method of Ferguson and Sellers[3] utilizing a closed circuit apparatus with eight chambers, each containing one rat. The apparatus was immersed in a water bath at 28.0° ± 0.5°c. The temperature in the chambers was 30.0° ± 0.5°c. Measurements were made at frequent

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§ Eli Lilly & Co., Indianapolis, Lot No. 258–234 B-54-2.

‖ Armour & Co. Ltd., Chicago, Illinois.

¶ Parke-Davis & Co., Brockville, Ontario, 1 Canada, cc. ampoules of adrenalin-in-oil 1:500.

** Merck & Co. Ltd., Montreal, Canada.

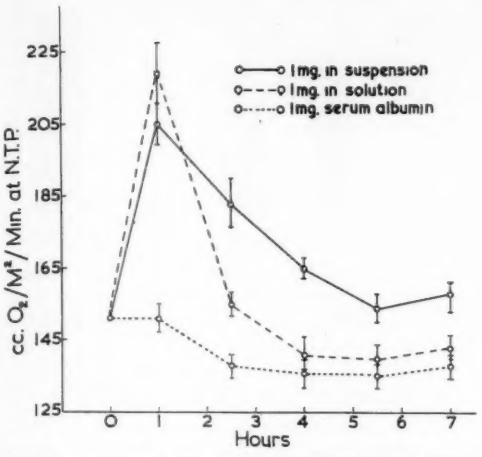

FIG. 1. Change in the oxygen consumption of normal rats following the subcutaneous injection of glucagon. Control rats were given an injection of albumin.

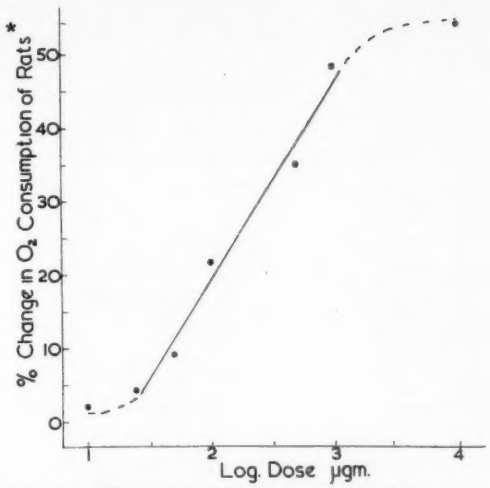

FIG. 2. Effect of glucagon on the metabolic rate; dose versus response.

* One hour after administration of glucagon solution.

intervals over a seven hour period. The volume of oxygen consumed by each rat per minute per square meter of body surface at normal temperature and pressure was calculated using Lee's formula.[4]

PROCEDURES AND RESULTS

The Effect of Glucagon and Adrenalin on Intact Rats

The metabolic rates of rats treated with a single injection of a glucagon solution or a glucagon suspension were compared with those of control animals treated with an injection of crystalline plasma albumin (Fig. 1). In each instance, glucagon produced a marked elevation of the metabolic rate. The maximum increase occurred one hour after the injection, but diminished rapidly thereafter. The mode of administration of the hormone affected the magnitude and duration of the response. The injection of glucagon dissolved in alkaline saline caused a greater increase (47 per cent) in the metabolic rate than glucagon given as a suspension in neutral saline (35 per cent). However, the suspension produced a more prolonged effect (Fig. 1); the rate was still elevated by 15 per cent at the end of seven hours. Additional experiments showed that over a range of 25 to 1,000 µg. of glucagon, a linear

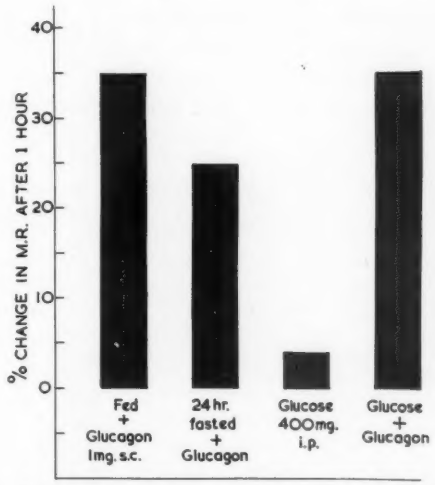

FIG. 3. Effect of glucagon and glucose on the metabolic rate of rats. No correlation between the hyperglycemic action of glucagon and its effect on oxygen consumption is evident.

relationship existed between the logarithm of the amount of glucagon administered and the percentage change in oxygen consumption (Fig. 2).

No correlation could be established between the hyperglycemic action of glucagon and its effect on oxygen consumption. When the rats were fasted for twenty-four hours the

TABLE I
Metabolic Rates of Rats One Hour After Adrenalin Administration*

Groups	No. of Rats	Metabolic Rate‡	Increase	per cent
Intact				
Control.....	10	143 ± 5		
Treated.....	10	213 ± 10	70	49.0
Adrenalectomized				
Control.....	15	146 ± 4		
Treated.....	16	171 ± 5	25	17.1
Adrenalectomized plus cortisone†				
Control.....	12	167 ± 9		
Treated.....	11	247 ± 14	86	47.9

* 0.1 mg. adrenalin-in-oil per rat.
† 2.5 mg. cortisone daily per rat.
‡ In this and other table, cc. O_2/M^2/minute at normal temperature and pressure ± standard error of the mean.

administration of glucagon did not produce a significant change in the blood sugar level but it did cause a 25 per cent increase in the metabolic rate (Fig. 3). This increase was 10 per cent less than that observed in the rats fasted four hours. Conversely, the intraperitoneal injection of 400 mg. of glucose (sufficient to produce a hyperglycemia of slightly greater

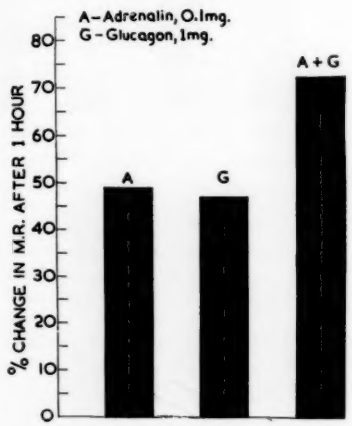

FIG. 4. Synergistic effects of adrenalin and glucagon in stimulating oxygen consumption of rats. The changes represented by bars A and G were the greatest that could be produced by injection of either of these hormones.

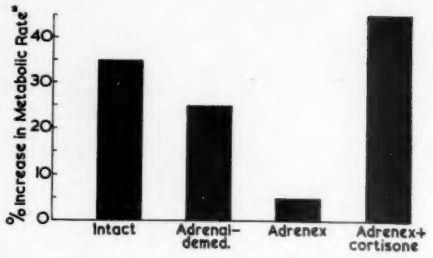

FIG. 5. Effect of adrenalectomy and adrenal-demedullation on the metabolic response to glucagon. The small increase shown for the adrenalectomized rats is not significant statitiscally.

* One hour after 1 mg. of glucagon was administered subcutaneously.

magnitude and duration than that produced by glucagon, increased the metabolic rate by only 4 per cent. It was also observed that the concomitant administration of glucose potentiated the hyperglycemia induced by glucagon but did not enhance its effect on metabolic rate (Fig. 3).

The effect of adrenalin on the metabolic rate of rats was studied under the same conditions used for the experiments with glucagon. It was found that adrenalin, like glucagon, was most effective one hour after administration (Table I).

An increase in oxygen consumption of 47 to 49 per cent appeared to be the limit of the change that could be elicited in intact animals with either glucagon or adrenalin, but when these hormones were administered together increases of 72 to 75 per cent were observed (Fig. 4).

Adrenalectomy and Adrenal-demedullation

The administration of glucagon to adrenalectomized rats sixteen to twenty-one days after surgery did not elicit a significant change in the metabolic rate (Fig. 5). Treatment with 2.5 mg. cortisone daily for eight to ten days not only restored but also potentiated the response to glucagon (Fig. 5). The magnitude of the response was closely related to the duration of cortisone therapy. Three to four days of treatment with this steroid were required before the metabolic rate of the adrenalectomized rats could be altered by glucagon. Full metabolic effect was not restored

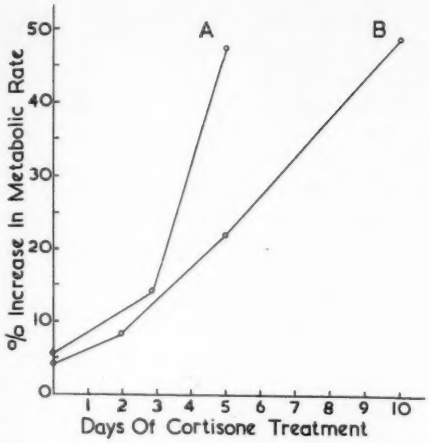

FIG. 6. Time required for cortisone therapy (2.5 mg. daily) to restore the metabolic response of adrenalectomized rats to glucagon. The per cent increase in metabolic rate refers to the change one hour after the injection of 1 mg. of glucagon in alkaline solution. Curve A represents animals that received injections of cortisone one day after adrenalectomy. Curve B represents rats that received injections of cortisone sixteen days after adrenalectomy.

until nine to ten days of cortisone treatment (Fig. 6, curve B).

The metabolic response to glucagon was immediately abolished by adrenalectomy and could not be maintained by starting cortisone injections the same day the suprarenal glands were removed. However, the immediate application of cortisone therapy restored the glucagon effect in a shorter time than when similar treatment was started sixteen days after the rats had been adrenalectomized (Fig. 6, curve A).

The administration of a single dose of glucagon increased the metabolic rate of adrenaldemedullated rats by 25 per cent in one hour. This response was 10 per cent less than that obtained in the intact animal (Fig. 5). At autopsy, the adrenal glands of these animals were found to be very small in comparison to the glands of intact animals of similar weight. A histologic examination of the glands revealed that the regenerated cortices were smaller than normal.

Adrenalectomy also reduced the response of the animal to adrenalin (Table I). The administration of a single dose of the hormone fourteen days after operation increased the metabolic rate of the rats by only 17 per cent. This response was a third of that obtained in intact animals. The daily administration of cortisone from the time of adrenalectomy (Table I) restored the effect of adrenalin.

Thyroidectomy

The metabolic rate of the thyroidectomized rats fell and then stabilized at a value 28 per cent below the metabolic rate of the intact control animals during a three week postoperative period. The administration of glucagon to these animals one month postoperatively did not alter the oxygen consumption significantly (Table II).

TABLE II

Effect of Glucagon on the Metabolic Rate of Thyroidectomized Rats

Group	No. of Rats	Metabolic Rate*			
		Hours after Injection			
		1.0	2.5	4.0	5.5
Intact†	24	151 ± 4	138 ± 3	136 ± 4	135 ± 3
Thyroidectomized					
Control†	15	109 ± 5‡	100 ± 3	108 ± 6	100 ± 3
Treated§	15	121 ± 5	108 ± 3	105 ± 2	107 ± 5

* See footnote (‡) in Table I.
† Injected with 1 mg. albumin dissolved in saline.
§ 1.0 mg. glucagon suspended in saline.
‡ The differences are not significant at the 5 per cent level of confidence.

COMMENTS

It appears that the effect of glucagon and of adrenalin on the metabolic rate is similar in some respects. The greatest increase in oxygen consumption obtained after a single injection of glucagon was about 47 per cent. It has been shown[5,6] that the maximum response to adrenalin is of a similar order of magnitude. The effect of both substances is rapid and transient. The metabolic rate was increased to the greatest extent one hour after a single injection of either of these hormones and returned to control levels during the next few hours.

The similarity in the patterns of response produced by the two substances suggested that the effect of glucagon on the metabolic rate might be due to the liberation of adrenalin. This does not appear to be true since the glucagon was obtained in adrenalectomized animals maintained on cortisone and in adrenal-demedullated animals. The observation that the response to both adrenalin and glucagon was greatly reduced by adrenalectomy, but was gradually recovered and even potentiated (Fig. 5; Table 1) by treating these animals with cortisone, suggests that the adrenal cortex plays an important role in the regulation of respiratory metabolism. The small reduction in the response obtained in the adrenal-demedullated animal compared to that observed in the intact (36 per cent) or the cortisone-treated adrenalectomized animal (47 per cent) may be explained by the decrease in total secretory capacity noted in glands which have regenerated after adrenal enucleation.[7]

The influence of adrenalin on respiratory metabolism, i.e., its "calorigenic action," has been ascribed to an increase in the production and utilization of glucose and lactic acid, an increased activity of skeletal muscle, a peripheral vasoconstriction leading to a rise in body temperature and subsequent increase in metabolic rate, and to the hormone acting as a respiratory catalyst at the cellular level.[8-10] Griffith[10] believes the calorigenic action is the resultant of the sum of these effects. Glucagon does not produce hyperlacticacidemia or cardiovascular effects[11-15] and there is no evidence of a stimulating effect on skeletal muscle. The only feature that glucagon and adrenalin have in common is the ability to produce hyperglycemia by stimulating hepatic glycogenolysis. Both Griffith[10] and Ellis,[9] after reviewing the literature, concluded that the hyperglycemia induced by adrenalin plays no significant role in its calorigenic action and, in the present investigation, no evidence has been obtained that glucagon-induced hyperglycemia is directly related to its effect on oxygen consumption.

Both adrenalin and glucagon are dependent on the presence of the thyroid for their calorigenic action. Swanson[5] found that adrenalin had no effect on the oxygen consumption of rats in the absence of thyroxin. Extending the findings of earlier workers,[6,16] she observed that an increase in the metabolic rate induced by adrenalin was directly related to the circulating level of thyroxin. This finding suggested that adrenalin increases the rate of production of substrate (lactic acid) while thyroxin increases its utilization. Thus the calorigenic effect would be limited not only by the level of circulating thyroid hormone but also by the availability of substrate.

This concept might be used to explain the relationship between glucagon, thyroxin and the metabolic rate. In this case the substrate could be the deaminated residues of amino acids. It has been shown that the administration of glucagon is followed immediately by an increase in the rate of urea synthesis and amino acid catabolism.[17,18]

It is clear that glucagon does increase the oxygen consumption of rats. The assumption that this respiratory change reflects an increase in the oxidation of body nutrients with a consequent increase in heat production is strongly supported by a previous investigation which showed that rats treated with glucagon grow more slowly than untreated animals consuming identical amounts of food.

SUMMARY

The administration of glucagon to intact rats increased their oxygen consumption by as much as 47 per cent. The change was greatest one hour after the administration of glucagon and decreased slowly thereafter. A linear relation-

ship was observed between the percentage increase in the metabolic rate and the logarithm of the dose of glucagon. No correlation could be established between the hyperglycemic action of glucagon and its effect on metabolic rate. The increase in oxygen consumption produced by glucagon was similar in magnitude and duration to the change affected by adrenalin. The administration of glucagon and adrenalin together caused a greater increase than that produced by giving optimal amounts of only one of these substances. The effect of glucagon on respiratory metabolism was abolished after thyroidectomy and adrenalectomy had been performed. Its action appeared to be independent of the adrenal medulla since oxygen consumption was stimulated in adrenal-demedullated rats and in adrenalectomized rats treated with cortisone. Adrenalectomy also diminished the effect of adrenalin on the metabolic rate by 60 per cent. The administration of cortisone potentiated the effects of both glucagon and adrenalin.

The significance of the similarity between the calorigenic action of glucagon and adrenalin are briefly discussed.

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DISCUSSION

Dr. Estelle R. Ramey (*Washington, D. C.*): Dr. Salter, does the chronic administration of glucagon maintain any of these phenomena? Past history with respect to the hyperglycemic effect, for example, indicates that continuous administration may show some kind of either adaptation or lack of effect with this hormone, and I wondered whether these other effects behave similarly.

Dr. Salter: We have administered glucagon to rats for periods up to six months, and we have seen no sign of any adaptation. The rats lose weight and stay thin, while their metabolic rate remains elevated.

We have administered glucagon for five to six months to rabbits, and in so doing have produced what is truly a "metaglucagon" diabetes. These animals have remained diabetic for as long as seventy days after the cessation of treatment with glucagon.

Occasionally a rabbit seems to develop an antibody to glucagon. These animals, of course, become completely resistant; however, this is rare.

Dr. G. C. Kennedy (*Cambridge, England*): I was struck by Dr. Salter's saying that there was a similarity between the effects of cortisone and of glucagon, because I have had exactly the same experience with high doses of cortisone on appetite and weight change. Has he tried the effect of glucagon treatment on rats with hypothalamic lesions?

The effect of cortisone on inhibiting the appetite is abolished if one punctures the hypothalamus and produces hyperphagia first, and it would be interesting, I think, to know whether there is any possibility that a similar hint of central action of glucagon could be elicited by using a hypothalamic obese preparation.

Dr. Salter: We have not done that, but Dr Stevenson and I have discussed it several times.

Dr. Theodore B. Van Itallie (*New York, New York*): Dr. Salter has implied that glucagon may have a direct effect on amino acid catabolism. While this may be true, I think it is important not to underestimate the fact that glucagon depletes the liver of glycogen and that a liver depleted of glycogen may in itself be a powerful stimulus to gluconeogenesis from amino acids.

In some studies performed by Dr. Shoemaker and myself, studying the flux of various metabolites across the dog liver in response to glucagon, we found that shortly after the administration of glucagon, with the release of considerable amounts of glucose from the liver, the liver began to remove appreciable amounts of alpha amino nitrogen from the blood. This would tend to support the notion that to some degree the drop in amino acid nitrogen would be due to liver uptake of amino acids with their transformation into new glycogen.

Dr. Salter: The drop in amino acid nitrogen cannot be due to anything else, because the urea excretion

and the blood urea rise markedly. I would like to emphasize again that this effect is striking.

With regard to the glycogenolytic action, in liver slices we have not been able to demonstrate an effect of epinephrine on urea synthesis, and we have considered the same possibility. Our original contention was that epinephrine was acting peripherally as well as hepatically, whereas glucagon only acts on the hepatic tissue. We thought that perhaps the feedback of lactic acid from the muscle was helping maintain the energy equilibrium in the liver. If this were true some of the effects of glucagon might be done by giving epinephrine coincidentally. But this does not happen. If epinephrine is administered along with glucagon, it does not modify its effect on blood amino acids.

Dr. Kenneth Crispell (*New York, New York*): You seemed to show that cortisone is permissive for this reaction because you used very small doses. Do you know if small doses of thyroid are permissive in the thyroidectomized animal?

Dr. Salter: Yes. The administration of thyroid in small amounts (5 or 10 gamma a day) will restore the metabolic rate response completely.

The permissive effect of cortisone is just on the metabolic rate, not on the amino acid metabolism.

Dr. John R. Brobeck (*Philadelphia, Pennsylvania*): Therefore, you do not have to suppose that there is an increased secretion of thyroxin to cause the increase in metabolic rate.

Dr. Salter: No.

Dr. Brobeck: If there is enough thyroxin there, glucagon will then cause the increase?

Dr. Salter: On a constant does of thyroxin, you get the increase.

Dr. Rachmiel Levine (*Chicago, Illinois*): Does insulin counteract the effect of glucagon on raising the metabolic rate? The reason I am asking is that depancreatized dogs, within twenty-four hours after pancreatectomy, have a high metabolic rate, which is diminished by insulin. I wonder whether the metabolic rate is due to some peripheral interference with carbohydrate metabolism and, therefore, the appearance of intermediates, raising the rate. Does insulin counteract this reaction?

Dr. Salter: I don't know. There was another point that I should have mentioned. We think that the increase in metabolic rate may be completely due to a change in oxygen consumption by the liver, even though it would mean a tremendous increase. We have tried at various times to detect a change in the temperature of the liver, but without success. Dr. Van Itallie and his group showed that the blood flow through the liver was greatly increased following glucagon administration, which could easily account for this failure.

If you give glucagon, take the liver out and incubate it *in vitro*, the oxygen consumption of the organ rises by as much as 150 per cent.

Dr. Jean Mayer (*Boston, Massachusetts*): The evidence for possibly increased glucagon secretion in obese hyperglycemic mice is almost entirely circumstantial: they have hypertrophy of alpha cells, an increased turnover of liver glycogen, increased phosphorylase activity of the liver, plus a reaction to alpha cell-destroying agents. On the other hand, the evidence that they have increased insulin secretion is now well documented. This may bear on Dr. Levine's comment, in that the increased pancreatic content of insulin has been shown, in particular at the Banting and Best Institute. Dr. Renold and his group have shown that there is also considerably increased circulating insulin-like activity.

It may well be that the action of glucagon is quite different in the presence of an excess of circulating insulin, and certainly some of the evidence presented by Dr. Elrich on the possible synergism of glucagon and insulin on carbohydrate metabolism may bear on that point.

I think the action of insulin is established while the action of glucagon is hypothetical.

The Hypothalamic Control of Gastric Hunger Contractions as a Component of the Mechanism of Regulation of Food Intake

JEAN MAYER, PH.D., D.SC.*

THE PROBLEM of regulation of food intake though relatively new as an experimental problem, is an old theoretical preoccupation in physiology. Eighteenth and nineteenth century physiologists debated the relative merits of the "central" theory, the "peripheral" theory and the "generalized" theory of hunger. However, the first solid experimental evidence concerning the physiology of hunger and the regulation of food intake was the study by Cannon and Washburne concerning gastric contractions in 1912.[1] Here was an objective, reproducible, measurable aspect of the problem. The success of Carlson's book[2] also testified to the relief of physiologists at being at last able to treat this important regulation as something else than a philosophical subject. It has since been shown by Quigley[3] and Grossman et al.[4] that hunger behavior and the time it took following a meal for sensations of hunger to return were not dependent upon the presence of the stomach or its innovation. Thus gastric hunger contractions are not necessary for the "control of hunger." At the same time, they are still an essential element of the hunger complex and any theory which purports to depict the over-all mechanism of regulation of food intake must be able to interpret the pattern of gastric contractions or else doomed in advance. It is my belief that the recent findings concerning the behavioral, metabolic and neurologic concomitants of gastric hunger contractions permit to integrate them with the over-all system of feedbacks which adjust food intake to energy output and storage.

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EXISTENCE OF GASTRIC CONTRACTIONS IN THE HUNGER STATE

Cannon and Washburne showed that in the fasted state special movements of the stomach known as "hunger contractions" can be demonstrated. These movements, and the sensations they produce, have been extensively studied by Carlson,[2] Quigley[3] and their associates. Hunger contractions can be: (1) seen in persons with gastrostromies; (2) felt by placing the hand on the abdominal wall; (3) seen as ripples of the abdominal wall of persons with diastasis of the recti; (4) recorded from an open tube which has been placed in the proximal end of the stomach; (5) seen fluoroscopically following the swallowing of barium sulfate by the outliner technic; (6) recorded from radiopaque clips fastened to the gastric serosa in order to outline the stomach; and (7) recorded by the balloon manometer technic.[3]

The contractions of hunger occur as a peristaltic wave usually involving the gastric fundus, the corpus, the antrum and the proximal duodenum. In man, weak peristaltic waves may originate midway in the stomach and progress to the distal antrum and the duodenum without involving the gastric fundus. A hunger wave of moderate intensity passes from the proximal to the distal end of the stomach in one to two minutes. As the waves are exaggerated,

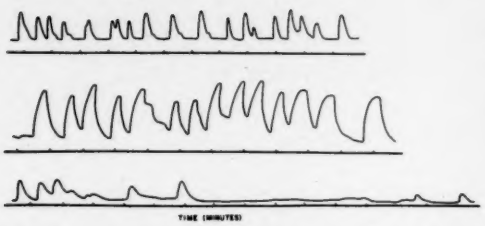


FIG. 1. Normal gastric hunger contractions in female rats weighing 300 gm.

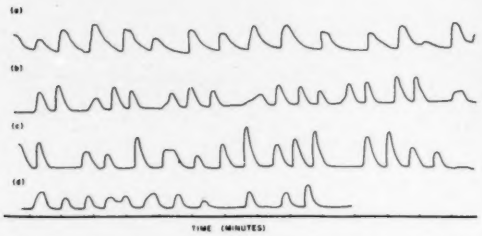


FIG. 2. Gastric hunger contractions in aphagic and adipsic female rats weighing 230 gm.: (a) twenty-four hours postoperatively, (b) forty-eight hours postoperatively, (c) seventy-two hours postoperatively and (d) before death on the fourth postoperative day.

they develop more frequently, progress more rapidly and are of greater magnitude, and the tonus is augmented. During vigorous activity, several waves may progress over the wall of the stomach simultaneously. As the periods of fasting increase, the periods of gastro-intestinal activity become more prolonged, shortening the periods of rest until the activity is nearly continuous. The tonus of the digestive tract is gradually increased so that the stomach and adjacent portions of the intestine remain partially contracted for long periods[3] (Fig. 1). The water-filled gastric balloon technic developed in our laboratory[5,6] shows gastric contractions with a swing of 5 to 15 cm. water in rats fasted four hours or more. The contractions have a general frequency of one or two per minute and form a regular and reproducible pattern. The fasting record shows a cyclic behavior both in amplitude and in frequency. This cyclic pattern becomes more pronounced until the end of the first twenty-four hours of fast and then remains constant for five days. This was the longest period studied[7] (Fig. 2).

FIG. 3. Top view of a hypothalamic mouse pressing lever in a Skinner-type box. Large round disc is part of an automatic feeder that discharges small pellets of food into tray (to right of mouse) whenever the mouse presses lever a predetermined number of times. To left of lever is tube from water bottle.

GASTRIC CONTRACTIONS AND HUNGER

In man, hunger contractions are accompanied by hunger sensations or hunger pangs as shown by Cannon[1] and confirmed by Carlson.[2] Like most visceral sensations, such hunger pangs are poorly defined and difficult to describe. However, the epigastric sensation, together with the feeling of emptiness and the disagreeable feeling of tension which usually accompany it, is unequivocally recognized as a hunger sensation by those persons who perceive it.

In experimental animals, sensations as such cannot be studied directly. It has been shown, however, in particular in our laboratory,[8] that it is possible to apply the recent behavioral technics of animals to the study of the regulation of food intake and thus to establish a correlation between central or metabolic abnormalities and hunger behavior. Figure 3 shows the box used for these studies. The pressing of a lever according to a predetermined schedule releases a food pellet of standard size. This schedule must be such that (1) the pressing of the lever will represent to the animal an amount of work sufficient for it to avoid unless it wants and will consume the pellet of food,

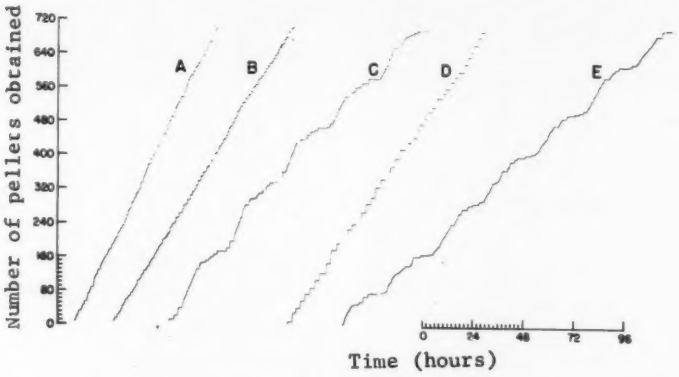


FIG. 4. Cumulative records of pellets obtained on a schedule of reward that allows one pellet of food for every twenty-five lever-pressing responses. Records A, B and C are from Swiss mice: A, goldthioglucose obese, B, hypothalamic obese and C, normal. Records D and E are from Ob ob littermates: D, hereditary obese hyperglycemic and E, normal. Note that the twenty-four hour cyclical changes in rate of feeding evidenced in records of normal mice, C, E, are either absent or barely discernible in the records of hyperphagic mice, A, B, D. (From: ANLIKER, J. and MAYER, J. An operant conditioning technique for studying feeding-fasting patterns in normal and obese mice. *Am. J. Physiol.*, 8: 667, 1956.[1])

and (2) the pressing of the lever does not entail work that will reduce the food intake from normal levels.

Figure 4 shows the cumulative food consumption pattern in different mice: C and E are normal animals; A and B are hypothalamic animals in which ventromedial hyperthalamic lesions have been induced by the stereotaxic technic[9] and by gold thioglucose, respectively.[10] The destruction of this part of the hypothalamus does not increase the rate of obtention of food (a behavioral measure of hunger) but instead decreases and even eliminates the long satiety period usually characteristic of the twenty-four hour intake pattern, a finding which demonstrates that the ventromedial area normally modifies food intake by acting as a brake and not as an accelerator.[11] The same technic, applied to rats in which intragastric balloons have been inserted permits the investigator to establish objectively a correlation between gastric contractions and hunger, as manifested by hunger behavior.[12]

Figure 5 represents a three day experiment (counted from noon to noon) during which time an animal with a permanently implanted balloon obtains food by pressing a lever. After a period of learning (first twelve hours), the animal seeks to obtain food whenever it experiences gastric contractions of sufficient intensity and duration. This demonstrates that such gastric contractions can be called, in fact, gastric hunger contractions and have probably the same significance in rats as they have in man. It is noteworthy that a slightly modified technic (using the pressing of a lever to deliver a known amount of a liquid diet into a gastric fistula) shows that the interpretation of gastric contractions is not dependent on taste nor even on gastric filling; the animals also learn to differentiate between solutions of saline and glucose or glucagon.[12]

GASTRIC CONTRACTIONS, METABOLIC EVENTS AND HUNGER IN MAN

The relationship between gastric contractions and glucose utilization in man has been studied recently by Stunkard and his co-workers.[13,14] The general findings are: (1) Gastric contractions and hunger sensations, as previously demonstrated, are concomitants (except in psychotic individuals). (2) Gastric contractions, like hunger sensations, occur or can be induced only when the rate of glucose utiliza-

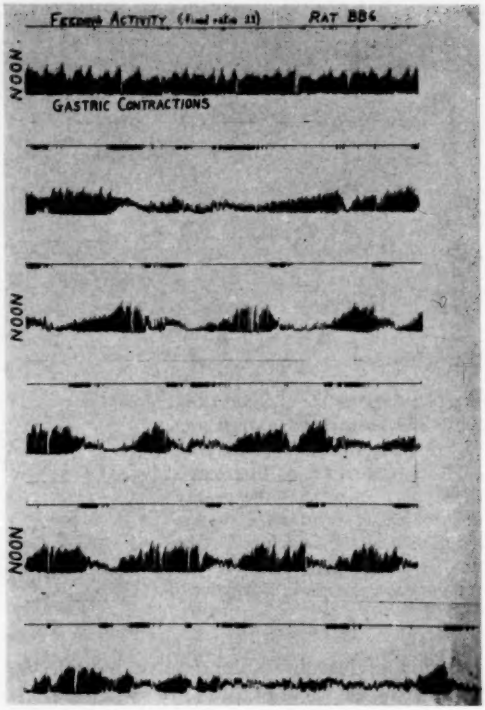


FIG. 5. Gastric contractions and feeding activity (lever pressing) on the first three days of study. The first twelve to fifteen hours represent a learning period. It is readily apparent that after that time the animal will seek to obtain food whenever it experiences gastric hunger contractions. (From: ANLIKER, J. and MORRISON, S. D. Unpublished results.[12])

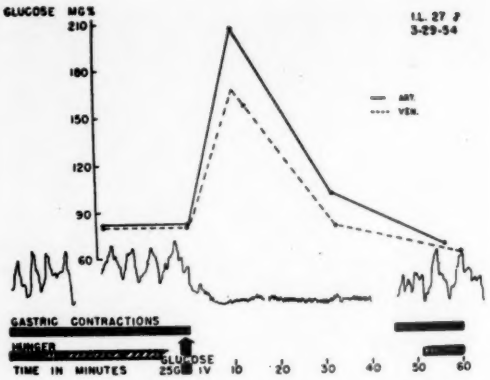


FIG. 6. Hunger feelings and hunger gastric contractions as affected by intravenous glucose infusions in a normal man. (From: STUNKARD, A. J. and WOLFF, H. G. Studies on the physiology of hunger. I. The effect of intravenous administration of glucose on gastric hunger contractions in man. *J. Clin. Invest.*, 35: 954, 1956.[13])

tion is reduced. For example, in the fasted state, when the difference in peripheral arteriovenous glucose is almost zero gastric hunger contractions are present or can be elicited readily by presenting the subject with food (the smell of food is particularly potent in inducing gastric contractions). Similarly, in patients with uncontrolled diabetes, gastric contractions can be eliminated bringing glucose utilization back to normal either by excessive amounts of food or insulin.

The effects of glucose are particularly interesting. In normal subjects whenever the infusion of glucose is followed by an increase in glucose utilization, gastric contractions are inhibited (Fig. 6). In patients with diabetics, glucose infusion is followed neither by glucose utilization or by decrease in gastric contractions (Fig. 7). In normal subjects, when an injection of glucose is administered (but not when it is ingested or released from the liver following the administration of glucagon), glucose utilization occasionally fails to increase. Gastric contractions do not stop in such cases. Failure to recognize that the cessation of gastric contractions is correlated with increase in glucose utilization and not with glucose levels, and that glucose injection is not necessarily correlated with increase in utilization is the cause of much confusion in the literature.

Because the events following glucose injection in man are not reproducible the findings of Stunkard, Van Itallie and Reiss[15] regarding the effects of administration of glucagon are of great interests.

The injection of 2 mg. of glucagon reproducibly eliminates gastric contractions (and hunger sensations) in human subjects. Figure 8 clearly shows that the elimination of gastric contractions lasts as long as glucose utilization proceeds actively and ceases when glucose utilization is reduced even though the absolute level of blood glucose may be still well above the fasting level.

One particularly impressive experiment with glucagon is related by Stunkard.[16] A patient

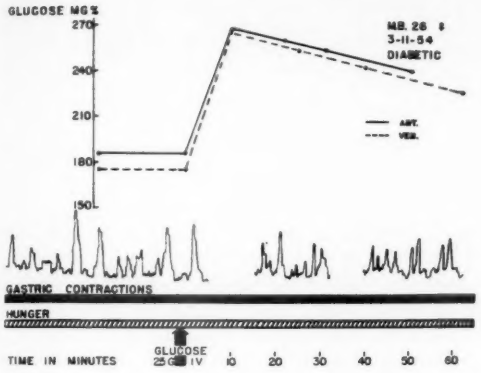


FIG. 7. Hunger feelings and hunger gastric contractions are not abolished by intravenous glucose infusions in a diabetic subject. (From: STUNKARD, A. J. and WOLFF, H. G. Studies on the physiology of hunger. I. The effect of intravenous administration of glucose on gastric hunger contractions in man. *J. Clin. Invest.*, 35: 954, 1956.[13])

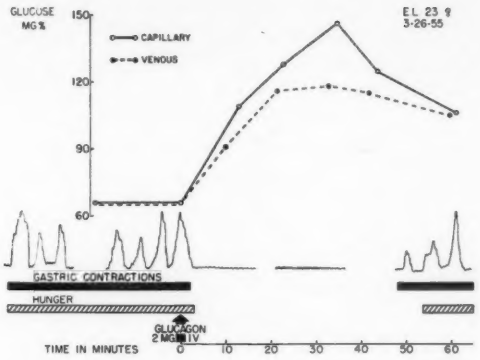


FIG. 8. Inhibiting effect of 2 mg. of glucagon on gastric hunger contractions and experience of hunger. As capillary-venous differences decrease, gastric hunger contractions return even though glucose levels are well above fasting values. The sample tracings are from a kymograph revolving at a speed of one inch per minute. Black bar represents actual time relations of the contractions to glucose levels and experience of hunger. (From: STUNKARD, A. J., VAN ITALLIE, T. B. and REIS, B. B. The mechanism of satiety: effect of glucagon on gastric hunger contractions in man. *Proc. Soc. Exper. Biol. & Med.*, 89: 258, 1955.[11])

who had lost practically all of his brain cortex in an accident was incapable of feeding himself. After one week of fasting he exhibited almost continuous gastric contractions. A variety of treatments, including infusion of amino acids and inducing of pyrexia (by rolling him in an electric blanket) did not inhibit gastric contractions. The only treatment with the exception for food which proved effective in inhibiting gastric contractions was the administration of glucagon.

GASTRIC CONTRACTIONS AND METABOLIC EVENTS IN EXPERIMENTAL ANIMALS

The sequence of metabolic events and gastric contractions in both normal animals and animals with hypothalamic lesions (ventromedial and lateral) has been studied in detail in our laboratory.[5–7]

Technics

The technics of recording hunger gastric contractions in man with air balloon systems are well known and have changed little since the experiments of Cannon and Washburne[1] and Carlson.[2] Quigley[3] has refined the methods and introduced the triple tandem balloon technic for more detailed studies of the portion of the stomach and the gastrointestinal tract from which hunger contractions originate. The experiment in animals which forms the basis of this review were conducted in young adult rats (Sprague-Dawley). The balloons were inserted permanently in the stomach by passing them through the musculature wall below the incision, down subcutaneously and finally bringing them out near the base of the tail. Terramycin® was sprinkled over the abdominal cavity and the operative areas and incorporated into the drinking water. During the immediate postoperative period, the rats were fed a mash. The recovery period from balloon insertion took about ten to fourteen days. Normal and hyperphagic rats were kept for three months and more after the insertion of balloons.

Hypothalamic lesions were induced with the Krieg stereotaxic instrument. (Hyperphagic lesions in females between planes 56 and 57, 1 mm. superior to the floor of the brain, 0.5 mm. off the midline from each side; current 2 ma. for 30 seconds; lesions considered successful if the daily weight greater than 1 gm. during the first twenty days following operation. Aphagic lesions made in animals with balloon already inserted, same plane, 2 mm. off the midline, same current; survival of completely adipsic-aphagic animals rarely exceeded four days.)

To obviate the many drawbacks of air filled systems, such as difficulties in standardization, presence of milevant fluctuations, and impossibility of differentiating between pressure and volume changes, a water system was used in the experiments summarized here. Both the balloon tubing and tambour were filled with water.

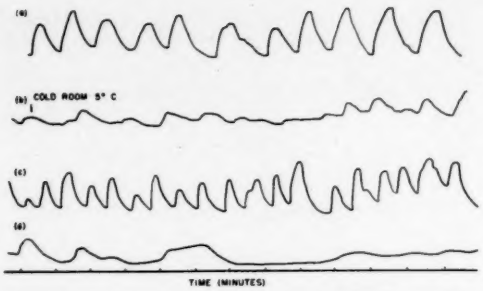


FIG. 9. Gastric hunger contractions in female rat weighing 275 gm. (a) before exposure to cold (5°C.), (b) after immediate exposure to cold (5°C.), (c) half an hour after exposure to cold (5°C.) and (d) before complete inhibition was observed.

Fasted Contractions

Two types of gastric motility are found in the records of fasted rats. The first is composed of a series of rythmic waves of constant amplitude without change in the base line pressure. The second type consists either of a series of rythmic contractions having a frequency and height similar to those of the first one but superimposed on an elevated base line, or a series of incomplete tetanic type of hunger contractions, again superimposed on an elevated base line. The frequency of contractions in both types is regular and about one or two per minute. The active contractions which follow one another in rapid succession go on for ten to fifteen minutes; they are then followed by a period of complete quiescence of periodic inhibition lasting for about five to ten minutes (Fig. 1).

Exposure to Cold and Heat

In the cold, a partial inhibition of gastric motility takes place immediately and lasts about ten minutes. There is progressive reappearance of normal hunger contractions followed after one to two hours by incomplete tetanic contractions. The height of the contractions then tends to decrease and the frequency shifts from one per minute to one per two minutes or less. This phase lasts for about half an hour and is followed by total cessation of contractions. (It is worth noting that milder exposure to cold at first not only eliminates gastric contractions but hunger behavior as well [Fig. 9]).

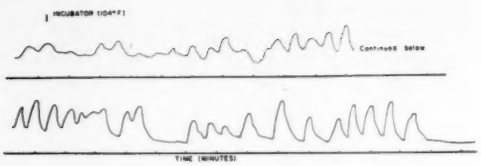


FIG. 10. Effect of heat (104°F. or 40°C.) on gastric hunger contractions in female rat weighing 330 gm., fasted overnight. The total absence of reaction (top) is typical; small reaction (bottom) takes place occasionally with 75 alpha, more frequently if dose is considerably increased.

Heat also inhibits gastric hunger contractions in fasted rats. During the first ten minutes in the warm room or incubator, the frequency of contractions increases from the normal rate of one per minute to two per minute; occurrence of tetanic types of hunger contractions with change in base line pressure is also noted. Then there is a periodic inhibition of gastric motility with decrease in the height of contractions and finally complete inhibition (Fig. 10).

Intravenous Materials

Intravenous injections of glucagon inhibits hunger contractions. At a dose of 50 μg., inhibition is about 30 per cent with 75 μg., 100 per cent (in a series of twenty-two consecutive tries). It is remarkable that the increase from 50 to 75 μg. is accompanied by such a difference in effects. Inhibition lasts on the average about one and five-tenths minutes with doses of 75 μg. In our experience, it always starts between forty-five seconds and sixty seconds following the administration of glucagon. The period of total inhibition of contractions is followed by a rapid succession of contractions with a rise in base line pressure. Intravenous injections of the same volume of water, physiologic saline solution or insulin diluting fluid, do not have any effect on gastric hunger contractions in the rat (Fig. 11).

At an interval of thirty seconds following administration of intravenous glucagon and before the inhibition of the gastric motility occurs in the animals, the glucose in the blood rises considerably from the control fasting

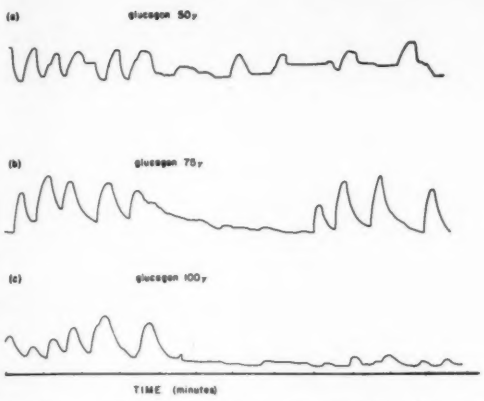


FIG. 11. Effect of intravenous administration of various doses of glucagon on gastric hunger contractions in female rat weighing 280 gm. (a) 50 μg. of glucagon, (b) 75 μg. of glucagon and (c) 100 μg. of glucagon.

values. At the same time, there is a drop in the level of inorganic phosphorus in the blood. Blood glucose continues to increase as inorganic phosphorus returns to the fasted level and gastric hunger contractions appear.[5–7]

Two units of insulin (Iletin®) administered intravenously produces gastric hunger contractions in fed animals. These appeared six to one hundred ten minutes (average thirty-one minutes) following the administration of insulin to a series of six animals. Glucagon does not inhibit insulin-induced gastric contractions unless the dose is increased to 100 μg. The inhibition produced by this dose of glucagon, following insulin administration, lasted, on the average, thirteen minutes in a series of six animals. The inhibitory period is longer than that produced by the same dose of glucagon without insulin in fasted animals.

Epinephrine hydrochloride in doses of 25 μg. inhibits gastric contractions in only 33 per cent of treated animals. The proportion is increased to 100 per cent when the dose is increased to 50 μg. Following the administration of epinephrine, inhibition of gastric contractions occurs within one to one and a half minutes and the period of inhibition lasts for about four minutes. Epinephrine has no effect on gastric tonus (as measured by the level of the base line) in spite of its effect in inhibiting hunger contractions. After epinephrine administration, the animals tremble and appear irritable. The appearance of returning gastric hunger contractions is similar to contractions following the infliction of pain in these animals. In fed animals pretreated with insulin, inhibition of gastric hunger contractions produced by 50 μg. of epinephrine is shorter than in fasted animals without insulin. While epinephrine has an hyperglycemic effect (30 per cent increase in one minute, 60 per cent in five minutes for 50 μg., norepinephrine, which has no such action, causes effects on gastric contractions which are indistinguishable from those of epinephrine administered at the same dose.

Comments

It is well known that cold increases the metabolic rate of the body and the utilization of carbohydrates[17] while heat decreases it. It is probable that the initial inhibition of gastric contractions in a cold environment is a reflex phenomenon, perhaps mediated by secretion of epinephrine. The mechanism of inhibition of hunger contractions after prolonged exposure to cold and after exposure to heat is not clear.

In experimental animals as in man, there is no correlation between absolute blood sugar levels and gastric hunger contractions. However, following the injection of glucagon, a decline in the inorganic phosphorus level of the blood indicates that an increase in the rate of glucose utilization takes place before the abolition of hunger contractions. The rise in inorganic phosphorus blood levels to its former value takes place before the reappearance of gastric hunger contractions. Thus, it appears that metabolic events induced by glucagon precede the gastric phenomena; this makes it possible for the effect of glucagon on glucose availability and utilization to be responsible for the effect on gastric contractions, a scheme the likelihood of which is considerably increased by findings on hypothalamic animals (*vide infra*).

The mutual interrelationship of the effects of insulin and glucagon is also of interest. It is well known that administration of insulin

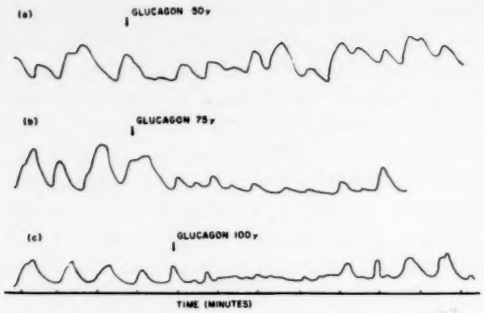


FIG. 12. Effect of intravenous administration of various doses of glucagon on gastric hunger contractions in a female rat weighing 250 gm. induced by 2 units of insulin. (a) 50 μg. of glucagon, (b) 75 μg. of glucagon and (c) 100 μg. of glucagon.

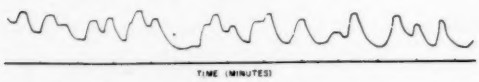


FIG. 13. Gastric hunger contractions in hyperphagic hypothalamic female rat weighing 450 gm.

to fed animals can induce hunger gastric contractions. This effect can be readily demonstrated in rats by the administration of two units of insulin (Fig. 12). However, inhibition of hunger contractions produced by 100 μg. of glucagon in fed animals pretreated with insulin is longer than that induced by the same dose of glucagon in fasted animals not treated with insulin. The inhibition of gastric contractions by this dose of glucagon in fed animals pretreated with insulin is also longer than that produced by the same dose of glucagon in fasted animals pretreated with insulin. Since glucagon acts, at least in part, by stimulating hepatic glycogenolysis, it is understandable that the action of glucagon in inhibiting gastric motility in fasted rats should be briefer than in fed animals with much greater hepatic glycogen reserves.

The synergistic action of insulin and glucagon in prolonging inhibition of gastric motility correlates well with the findings of Elrick et al.[18] who demonstrated that simultaneous administration of insulin and glucagon causes a significantly greater increase in peripheral glucose utilization than administration of either one alone. While epinephrine is known to have the effects of carbohydrate metabolism (including the hyperglycemia noted in the experiments summarized here), the fact that norepinephrine, which has no such effects, also inhibits hunger contractions in fasted rats when given at the same dose level as epinephrine shows that the effect of the latter to its local action upon the gastrointestinal tract. In spite of superficial similarities in the effects of the administration of glucagon and epinephrine, it appears that the ways in which these hormones inhibit gastric hunger contractions are radically different from each other.

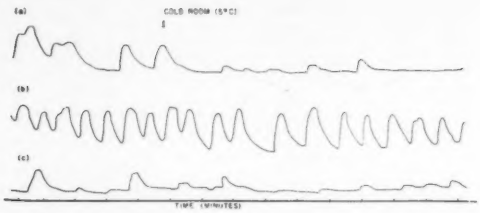


FIG. 14. Gastric hunger contractions in hyperphagic hypothalamic female rat weighing 500 gm. (a) after immediate exposure to cold (5°c.), (b) after half an hour in a cold room (5°c.) and (c) prolonged exposure to cold.

HYPOTHALAMIC HYPERPHAGIC ANIMALS

Hypothalamic Control of Gastric Contractions

Figure 13 shows a typical record of the gastric hunger contractions observed in hypothalamic rats fasted overnight. Hyperphagic rats (twenty-four rats were studied carefully in this respect) show no significant difference in their pattern of fasting contractions from that seen in normal rats. Neither the amplitude nor the frequency of hunger contractions in these rats is greater than those observed in normal animals. The only difference noted is that tetanic contractions with a change in base pressure appear somewhat more frequently in their records even through hypothalamic rats fasted only a few hours.

Exposure to Cold and Heat

Total inhibition of gastric contractions occurs in hyperphagic rats on the average five and one half hours after exposure to cold (5°c). This is more than twice as long as with normal animals. The patterns of gastric activity in

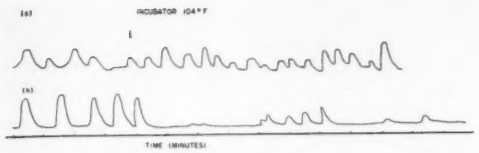


FIG. 15. Gastric hunger contractions in hyperphagic hypothalamic female rats weighing 425 gm. following (a) immediate exposure to heat (104°F. or 40°C.) and (b) twenty minutes in an incubator (104°F. or 40°C.).

these animals are the same as those observed in normal rats (Fig. 14). After sudden exposure to cold, gastric hunger contractions disappear for fifteen to twenty minutes. During most of the time in the cold room, the incomplete tetanic type of gastric motility with a changed base line dominates the picture. The height of contractions remains the same as that recorded in a thermoneutral environment. (Again it is worth noting that an immediate effect of cold, besides elimination of gastric hunger contractions, is elimination of hunger behavior.)

In normal animals and hyperphagic rats, sudden exposure to heat inhibits gastric hunger contractions. The frequency and sometimes the height of contractions increase after exposure to a higher environmental temperature. Contractions are then periodically inhibited with a decrease in height before complete inhibition supervenes (Fig. 15).

Intravenous Materials

Administration of 75 μg. of glucagon intravenously almost invariably fails to produce complete inhibition of hunger contractions in animals with lesions of the ventromedial nuclei, whether the animals are allowed to become and remain obese or whether reduced to their preoperative weight after demonstrating their hyperphagia. Inhibition failed to occur in 80 per cent of the animals (by comparison with 100 per cent inhibition in nonoperated animals). In the few animals in which this dose has an effect, the effect is usually slight (Fig. 16).

Intravenous injection of 50 μg. of epinephrine is effective in inhibiting gastric hunger contractions in hyperphagic hypothalamic rats. The period of inhibition of gastric

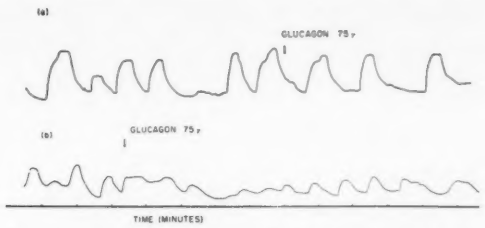


FIG. 16 Effect of intravenous administration of 75 μg. of glucagon on hunger gastric contractions in aphagic hypothalamic female rat weighing 250 gm. (a) Typical absence of effect or gastric contractions and (b) occasional partial inhibition.

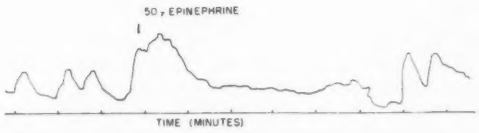
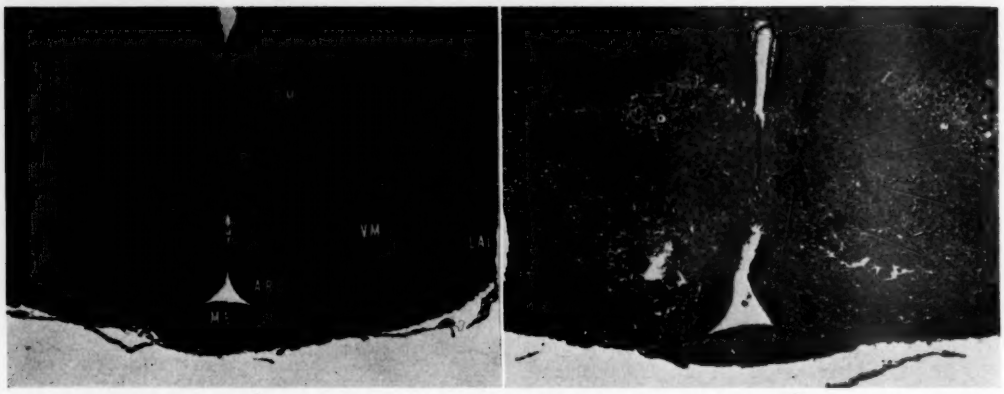


FIG. 17. Effect of intravenous administration of 50 μg. of epinephrine on gastric hunger contractions in hyperphagic hypothalamic female rat weighing 320 gm.

hunger contractions following the injection of this dose of epinephrine ranges from one and one half to seven minutes, with an average of five minutes (Fig. 17). Norepinephrine is equally effective. Thus the failure of the animals to respond to glucagon is not due to a failure of the stomach to respond to inhibitory material.

HYPOTHALAMIC APHAGIC ANIMALS

Gastric hunger contractions in hypothalamic aphagic animals do not differ from those seen in normal animals deprived of food and water for an equivalent period of time. On the first postoperative day, the frequency of hunger contractions is one or two per minute. Each contraction follows the other rapidly without any change in base line pressure. On the second and third postoperative days, the picture remains the same, except for some rats in which a tetanic type of hunger contraction appears. Before the death of the animals (fourth to sixth day), periodic inhibition of gastric hunger contractions with reduction in amplitude is observed. Normal rats deprived of food and water show the same sequence.



A B

FIG. 18. Photomicrographs (× 65) taken of hematoxylin and eosin stained sections through tuberal or infundibular region of the hypothalamus showing the same area in three different animals. A, section through hypothalamus of a goldthiomalate treated mouse, indicating a normal hypothalamic nuclear configuration and containing a normal number of neurons. Photomicrograph is labeled to indicate normal landmarks and nuclei seen in this region of the hypothalamus: DM, dorsomedial nucleus; P, periventricular region; V, third ventricle; VM, ventromedian nucleus; LAT, lateral hypothalamic area; ARC, arcuate nucleus; ME, median eminency. B, same area as in Figure 18A of hypothalamus of mouse three days after the administration of goldthioglucose. Note marked loss of neurons and presence of pyknotic cells in ventral part of hypothalamus. Normal hypothalamic nuclear pattern has been lost. Arrows on right side of photomicrograph point to punctate hemorrhages. Arrow on left points to line of demarcation between area of edema (ventral) an normal tissue (dorsal). (From: MARSHALL, N. V., BARRNETT R. J. and MAYER, J. Hypothalamic lesions in goldthioglucose injected mice. *Proc. Soc. Exper. Biol. & Med.*, 90: 240, 1955.[10])

Exposure to Cold and Heat

The response of aphagic animals to heat and cold is identical to that seen in normal animals fasted for an equivalent period.

Intravenous Materials

Unlike hyperphagic rats, a dose of 75 μg. of glucagon inhibits gastric hunger contractions in all aphagic animals, with an inhibition period of one and one half to three minutes. Inhibition normally occurs within one minute after the injection of glucagon. As in normal animals, a rise in blood glucose occurs before the inhibition of gastric hunger contractions. The effect of epinephrine and norepinephrine is the same as has been seen in normal animals.

GLUCOSTATIC MECHANISM AND GASTRIC HUNGER CONTRACTIONS

Thus it appears that gastric hunger contractions are essentially similar in normal, hypothalamic hyperphagic and hyperthalamic aphagic rats. It is evident that the complete reduction of food intake in animals with lateral lesions is not due to the failure of gastric hunger contractions to appear or to respond to various stimuli. Their gastric responses to fasting and to cold, heat, glucagon and epinephrine are entirely normal. Their response to glucagon is particularly remarkable in that it takes place in spite of the low levels of glycogen present after a prolonged fast. Thus it is apparent that while destruction of the lateral hypothalamus renders the animal insensitive (or at least unreactive) to the stimuli of hunger (and thirst), the lateral hypothalamus does not appear to exert any direct influence upon gastric hunger contractions.

The role of the ventromedial area is quite different. Destruction of this area eliminates the response of gastric hunger contractions to glucagon in spite of the normal or greater than normal metabolic response. It also considerably delays the long term response to cold. That such a lack of response is not due to inability of the gastric contractions to be inhibited is shown by their normal reaction to epinephrine, norepinephrine and heat.

TABLE I
Survival and Incidence of Obesity Following the Administration of Goldthio Compounds in a Representative Series

Compounds	Body Weight (mg./gm.)	Number of Mice Injected	Number of Mice Surviving	Number of Obese Mice
Goldthioglucose ($AuSC_6H_{11}O_5$) Gold content 50%	0.50	50	42	2
	0.75	325	162	51
	1.00	710	379	235
	1.50	240	107	27
	2.00	57	7	2
Goldthiomalate ($AuSC_4H_3O_4Na_2$) Gold content 50%	0.20	18	16	0
	1.00	121	80	0
	1.50	38	6	0
	2.00	4	0	0
Goldthioglycoanilide ($AuSC_6H_5NHCOCH$) Gold content 54%	0.80	77	77	0
	1.50	20	20	0
	6.00	26	26	0
Goldsodium thiosulfate [$AuNa_3(S_2O_3)_2.2H_2O$] Gold content 37%	0.25	25	25	0
	0.50	20	7	0
	1.00	5	0	0
Goldthioglycerol ($AuSCH_2CHOHCH_2OH$) Gold content 65%	0.8	31	31	0
Goldthiocaproic acid [$AuS(CH_2)COOH$] Gold content 57%	0.9	33	33	0
Goldthiosorbitol [$AuSCH_2(CHOH)_4CH_2OH$] Gold content 50%	0.5	10	2	0
	1.0	25	0	0
	1.0	60	4	0
	1.5	25	0	0
Goldthiogalactose ($AuSC_6H_{11}O_5$) Gold content 50%	0.50	50	43	0
	1.00	150	133	0
	1.50	50	38	0

Destruction of the ventromedial hypothalamic area rendering the gastric contractions unresponsive to glucagon can be interpreted in the light of the studies reported previously on the mode of action, and the timing of the action, of glucagon. Response of gastric contractions in normal animals is not instantaneous. It takes place after glucose utilization has been considerably increased by glucagon (and, by the same token, ceases when glucose utilization has reverted to the normal rate). The logical explanation is that response to glucagon has been abolished because an area sensitive to the metabolic effects of glucagon has been eliminated.

THE VENTROMEDIAL HYPOTHALAMIC AREA AS A GLUCORECEPTIVE AREA

Circumstantial evidence suggesting that the ventromedial area is a glucoreceptive area has been reviewed previously.[19–21] Recently, much more cogent indications have been obtained. Marshall, Barrnett and I[22,23] have shown that goldthioglucose, but not other goldthio compounds of similar toxicity, produces destructive lesions in the ventromedial hypothalamic area. These lesions are most severe in the ventromedial area but also involve the ventral part of the lateral hypothalamic area, the arcuate nucleus and the median eminence (Fig. 18). Goldthiosorbitol does not cause such lesions (nor does it produce obesity) in spite of the structural similarity between glucose and sorbitol. Similarly, compounds in which gold is linked by a sulfur bridge to normal metabolites other than glucose, such as malic acid, caproic acid, galactose or glycerol do not induce hypothalamic lesions or obesity (Table 1). Hypothalamic lesions produced by gold-

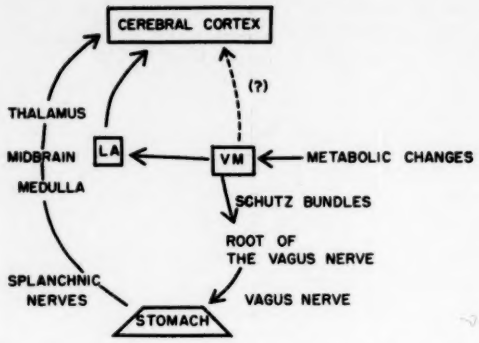


FIG. 19. Integration of the hypothalamic metabolic and gastric factors in the phenomenon of hunger. VM and LA stand for the ventromedial hypothalamic area and the lateral area of the hypothalamus, respectively.

thioglucose can be induced in the rat as well as in the mouse; other goldthio compounds, e.g. gold thiogalactose, goldthioglycerol, goldthiomalate, etc., cause no lesions in the rat. (Rats are more sensitive to gold toxicity than mice and do not survive the treatment; thus while the lesions can be induced by goldthioglucose, obesity cannot.) It appears that gold is drawn into the ventromedial hypothalamic cells, which it destroys, because of the peculiar affinity for glucose displayed by these cells. (Hypothalamic lesions induced by goldthioglucose are much more "purely" hyperphagic than lesions induced in the same area by electrocoagulation.) Animals who develop obesity from the administration of goldthioglucose do not display to the same degree associated disturbances such as gonadal dysfunction as seen in animals made obese through hypothalamic lesions obtained by electrocoagulation. For example, we have observed animals who develop obesity from administration of goldthioglucose to mate, carry and nurse normal litters. The histologic figures of lesions induced by goldthioglucose also confirm that functional localization is not limited to a given nucleus but that greater or lesser densities of cells sensitive to goldthioglucose (glucoreceptive) are to be found throughout the ventromedial hypothalamus interspersed with cells insensitive to goldthioglucose presumably performing functions other than the regulation of food intake.

Forssberg and Larsson[24] showed that this area had a highly atypical phosphate and glucose metabolism. More recently Larsson, working with Chain,[25] confirmed that the carbohydrate metabolism of this area was atypical, particularly in regard to the effect of potassium on glycolysis. The significant findings by Anand earlier in this symposium[26] demonstrating that glucose and insulin administration brings about reproducible change in the electrical activity of the feeding centers, also bring about conclusive evidence in favor of the glucoreceptive character of the centers.

Destruction of the ventromedial hypothalamic area eliminating sensitivity to the metabolic action of glucagon also confirms the glucoreceptive character of this area. The view previously advanced by this author that passage of glucose (or more probably of potassium iron associated with glucose phosphate) modifies food intake ("regulates" it, i.e. adjusts intake to energy output) by modulating the brake effect of this area on the more or less constantly activated lateral area, appears to be only part of the story, in view of the other role of the ventromedial area in regulating gastric hunger contractions.

THE VENTROMEDIAL AREA AS A GLUCORECEPTIVE REGULATOR OF GASTRIC HUNGER CONTRACTIONS

The mechanism of peripheral gastric hunger must be in part self-regulating. This is substantiated by the comparatively close agreement in the spontaneous motility of the Heidenhain pouch and the main stomach noted by Robins and Boyd,[27] the denervated Heidenhain pouch and the main stomach by Bercovitz,[28] the transplanted completely denervated pouch by Farrell and Ivy,[29] and several completely denervated gastric preparations by Quigley, Zettleman and Ivy.[30] At the same time, it appears that there is a supererogatory control of gastric contractions by the ventromedial area of the hypothalamus, itself attuned to the availability of carbohydrates in blood and hence to the over-all regulation of metabolism. There is an anatomic basis for such a mechanism (Fig. 19). The existence of the Schutz bundle relating the ventromedial area

to the roots of the vagus makes the following scheme acceptable: Decreased available glucose stimulates glucoreceptors in the ventromedial area of the hypothalamus. Hypothalamic glucoreceptors in turn originate nervous impulses traveling via the Schutz bundle to the roots of the vagus and through this nerve, influencing gastric hunger contractions. Gastric hunger contractions in turn give rise to afferent impulses carried by the splanchnic nerve to the medulla and on to the midbrain and the hypothalamus where they are relayed and finally integrated and interpreted as hunger sensations by the cerebral cortex. At the same time, the ventromedial area also modulates the lateral hypothalamic area which sends impulses to the cortex. An alternative pathway which connects the ventromedial area directly to the cortex has not been confirmed (even though a projection from the posterior part of the orbital surface of the cortex to the ventromedial and paraventricular nuclei of the hypothalamus has been reported in monkeys and men).[31] At this point, however, there is no physiological proof of such a direct connection.

SUMMARY

Modern behavioral methods make it possible to study gastric contractions in relation to hunger behavior in animals as well as in man. It can thus be shown that the significance of these contractions is probably the same in the rat as it is in man.

Gastric hunger contractions appear to be, at least in part, under hypothalamic control. One of the areas involved is the ventromedial area which has been shown to be sensitive to the metabolic state of the individual. One of the factors in this control may be the rate of glucose utilization by the ventromedial area of the hypothalamus.

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DISCUSSION

DR. C. N. H. LONG (*New Haven, Connecticut*): The idea that there are elements in the central nervous system that are dependent upon insulin in order to effect the metabolism of glucose is certainly new and, if true, would be an answer to the question as to why a diabetic animal is hungry.

If this is so, then we should regard these centers, in the absence of insulin, as being deprived of what we regard as the essential foodstuff for their operation. Therefore, I would think that we might expect to find an irritability of these centers, such as we find when we deprive them of glucose by the administration of excessive insulin. It would seem to me that the effect would be the same if the glucose is removed from the circulating blood or is prevented from entering the cells, as it would be if this hypothesis is correct and follows what we know about insulin in muscle.

I do not know that there is any evidence that in the animal with diabetes there is hyperexcitability, but still it may well be that, as Dr. Mayer has suggested, we have in this area neurons with different characteristics from those in the rest of the nervous system.

DR. GEORGE F. CAHILL, JR. (*Boston, Massachusetts*): If one takes a rat with diabetes and administers insulin, the hyperphagia continues for four or five or six days, and the animal blows up with obesity, has a tremendous A-V glucose difference, and still continues to eat.

If this sensitive center that is controlling the appetite were responsible for the hyperphagia, I should think insulin would cause cessation of the appetite in this animal within a few hours, not several weeks.

DR. JEAN MAYER: This question is difficult for me to answer, because in our experience we have not observed that. We believe that these centers need insulin to function but that once there is some insulin present, then they are, if anything, more sensitive to the level of blood glucose.

We observed lags, in many cases, in shifting about insulin, as you did, but I believe that they were in the opposite direction.

DR. ALBERT RENOLD (*Boston, Massachusetts*): If goldthioglucose accumulates in the ventromedial center because of the glucose component, and if the center is insulin-sensitive with regard to glucose, would it follow that one should increase the toxicity for goldthioglucose by the administration of insulin and have a decreased toxicity in the diabetic?

DR. MAYER: We have no data on animals with diabetes, but we have noted some increase in obesity with insulin.

The difficulty is that we also get a considerable increase in toxicity to gold compounds in general if we administer a toxic compound and at the same time give insulin. Although the results were favorable to the idea, the number of survivors were, we believe, too small to be conclusive.

On the other hand, if you administer enough glucose at the same time goldthioglucose is administered, a decrease in incidence of obesity is seen; and if sodium thioglucose is administered at the same time, the toxicity and the obesity can be almost eliminated.

DR. JAMES SALTER (*Toronto, Ontario, Canada*): We have found, particularly with protamine insulin, that the hyperphagia disappears extremely rapidly, within hours.

Did you give regular insulin, Dr. Cahill?

DR. CAHILL: I am quoting the experiments of Dr. Spiro, done in the laboratory of Dr. J. T. Hasting. These experiments were published in the "*Journal of Biological Chemistry.*" Two units of NPH insulin were administered every twenty-four hours. Whether it was given in one or two doses, I do not know. These animals continued to injest two or three times the normal amount of food for five days, during which time their body lipid increased to levels of 20 to 30 per cent. Only after that time did their increased appetite gradually come back to normal. They lost weight on the same dose of insulin, and returned to normal, at least as far as body composition was concerned.

Dr. Richard Field in Boston has shown that peripheral nerve is insulin-sensitive by demonstrating that, after a nerve is sectioned and Wallerian degeneration is obtained, the tissue loses its insulin sensitivity.

DR. SALTER: Dr. Mayer, what you think of the observation, which you have probably made yourself, that if glucagon is administered to a rat that has had insulin at the same time, even though its blood sugar level may be in the preconvulsive range, (35 to 40 mg. per cent) the animal still will not eat? This rat goes into convulsions, and his appetite is not affected.

DR. RACHMIEL LEVINE (*Chicago, Illinois*): What happens to the A-V difference for glucose in other areas when a depancreatized animal is hypophysectomized, at which time we know that a hyperphagia disappears?

DR. MAYER: I have not done any experiments on such animals. In animals which are both alloxan-treated and hypophysectomized—in which it is relatively easy to maintain hyperglycemia, with fewer injections than one would administer to normal animals, a drastically decreased food intake can be seen, but we did not do any measurement of glucose utilization.

DR. JOHN R. BROBECK (*Philadelphia, Pennsylvania*): Liebelt and Perry reported that goldthioglucose causes lesions in other parts of the brain. Dr. Mayer, how specific is this for the ventromedial nucleus and what does it do to the brains of animals such as rats, for example, in which it apparently does not selectively destroy this one region?

Dr. MAYER: This aea of the brain is destroyed.

DR. BROBECK: Alone?

DR. MAYER: The animals die, they also die following the same dose of goldthiosorbitol or goldthiogalactose but do not show brain lesions. I do not think that this is necessarily the only area of the brain which is particularly receptive to glucose. I think there is a great deal of circumstantial evidence, particularly the experiments of Duner and others on the effect of hypoglycemia on hydrochloric acid secretion in the stomach, which suggests that there may be a number of areas in the brain which have particular sensitivity to glucose and to goldthioglucose. The fact is that this is an area, in which many cells are destroyed with the animals becoming hyperphagic following goldthioglucose, and not following other goldthio compounds.

DR. RUSSELL J. BARRNETT (*New Haven, Connecticut*): The problem may be actually the blood brain barrier, which is fairly rigid. Although various leaks exist in the blood brain barrier, I prefer to think of them as buttered spots, such as the area postrema, the intercolumnar tubercle, in all the sections of the brain that I have examined (and these are sections through the hypothalamus and the rest of the brain included) I have never seen any lesions in any portion of the brain other than the hypothalamus.

The lesions are not restricted, though, to the ventromedial nucleus. The lesions are quite massive and actually extend from the optic chiasma through to the mammillary body and involve the entire central portion. The dorsal portion of the hypothalamus is usually spared in this. The lateral portion is not as much involved as the medial portion.

DR. MAYER: The lesions are widespread but they appear to be selective. For instance, it is well known from the work of Dr. Stevenson, and others, that there are all sorts of disturbances in functions other than food intake in animals in which lesions have been induced with a stereotaxic instrument, by contrast animals made obese with goldthioglucose seem to be only hyperphagic. They breed, have normal litters, and nurse the young, which does not happen in hypothalamic animals.

I think this fortifies the concept of Hess of there not being discrete nuclei but there being greater concentration of certain types of cells within a given area although the same cells are present in other parts of the hypothalamus.

Satiety Signals

MORTON I. GROSSMAN, M.D., PH.D.*

THE TITLE of this paper is a concession to brevity; a more exact one might read "Effects Produced by Eating which Inhibit Further Eating." Satiety is defined as an affective (hence, psychic) state brought on by eating and characterized by lack of desire to eat. So defined, it is subject to study only in man and only by interrogation. This paper deals with the behavioral correlate of satiety, cessation of eating. The study is based on observations on feeding behavior in animals, and it is doubtful whether inferences about the psychic state of satiety can be made from such data. Presumably, but not necessarily, when a man or animal stops eating while more food is available, he experiences satiety.

Eating leads to cessation of eating. What are the mechanisms of this effect? By fractionating the process of eating and its sequelae into stages, an assessment can be made of the contribution of various factors to the effect on cessation of eating.

SHAM FEEDING

Analysis of the fractionated feeding process begins with a consideration of sham feeding, in which the food eaten is diverted to the outside of the body through an esophageal fistula and thus is prevented from entering the stomach. In dogs so prepared the eating process is remarkably altered. Janowitz and Grossman[1] found that dogs whose mean duration of eating had been 2.5 minutes before making the esophageal opening ate for a mean of 14.1 minutes in the first sham feeding test to which they were subjected. Not only was duration of eating greatly prolonged in any one test, but this prolonged eating could be repeatedly demonstrated at short intervals. A second sham feeding test, an hour after the first one, lasted as long as the first test, and this process could be repeated hour after hour. Clearly, passage of food through only the mouth and pharynx is relatively ineffective in producing cessation of eating and the cessation so produced is shortlived.

What happens when an attempt is made to synthesize the normal eating process by placing food in the stomachs of dogs with esophagostomies? Some experiments by Share et al.[2] are summarized in Figure 1. Two effects are demonstrated. First, duration of sham feeding eighteen hours after the last intragastric feeding is inversely related to the size of the intragastric feeding. This effect occurs only after a period of equilibration over a number of days and is related to phenomena which will be considered later. Second, duration of sham feeding is reduced by an immediately preceding intragastric feeding. Because of interaction between the first and second effects the exact quantitative relations of the second effect is not certain. Specifically, it is not known whether a dog with an equilibrium of energy will show an exactly compensatory decrease in sham feeding as the result of an immediately preceding or simultaneous intragastric feeding. The next series of studies, on intragastric feeding in intact dogs with gastric fistulas, relates to this question.

INTRAGASTRIC FEEDING

Figure 2 presents data gathered by Janowitz and Grossman.[1] When about one fifth of the mean amount of food eaten during a long control period was placed directly into

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the stomach twenty minutes before feeding, no significant reduction in food intake occurred. When the fraction placed in the stomach was one-half the mean control intake, an apparently fully compensatory decrease was seen. A non-nutritious bulk substance (karaya gum gel) produced an effect indistinguishable from that of food. When the interval between intragastric feeding and offering food was increased to four hours, no depression of intake resulted. The results of this set of experiments suggest: (1) the threshold for detection of inhibition of oral feeding by intragastric feeding lies between one fifth and one half of the usual meal size; (2) inert bulk is as effective as food in producing inhibition, thus, a major signal for cessation of eating is elicited by gastric distention; (3) food which has emptied from the stomach does not produce inhibition. This last conclusion is valid only for short-term observations. These conclusions are based upon relatively few observations; the subject would repay more detailed study to establish the quantitative relations more clearly.

In addition to distention, another mechanism operating from the upper gastrointestinal tract which might be expected to influence feeding is the hormone, enterogastrone. Since this hormone inhibits gastric contractions and since the presence of gastric contractions is one of the indices of the hunger state, inhibition of these contractions by enterogastrone could be expected to be associated with inhibition of feeding. Janowitz and Grossman[3] tested this hypothesis in a study summarized in Figure 3. Prefeeding small amounts of sucrose or cream, amounts known to be capable of stopping gastric contractions by release of enterogastrone,[4] did not alter the amount of food eaten. Larger amounts of sucrose or cream were effective but this could be ascribed to gastric distention. These results suggest that enterogastrone does not play a role in regulation of food intake, but, as will be pointed out later, negative experiments are seldom crucial.

BLOOD LEVEL OF NUTRIENTS

The level of nutrients in the blood is increased

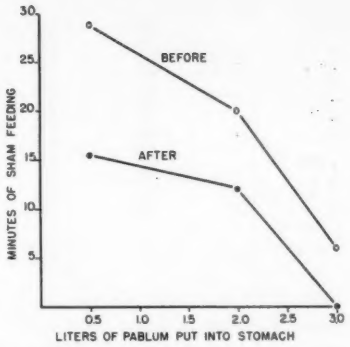

FIG. 1. Relation between size of intragastric feeding and duration of sham feeding. The upper line, labeled before, refers to tests performed before the last intragastric feeding. The lower line, labeled after, refers to tests performed immediately after intragastric feeding of the indicated amounts. Values are means of three dogs. (Data from: SHARE, I., MARTYNIUK, E. and GROSSMAN M. J. *Am. J. Physiol.*, 169: 229, 1952.[2])

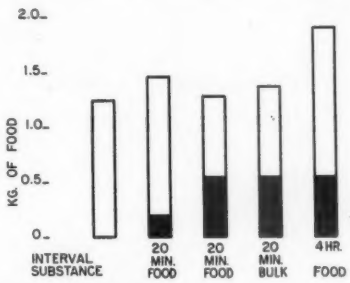

FIG. 2. Effect of putting substances in stomach on food intake immediately thereafter. Values are means for three dogs. Open bars represent oral food intake, black bars intragastric feeding. (Data from: JANOWITZ, H. D. and GROSSMAN, M. I. *Am. J. Physiol.*, 159: 143, 1949.[1])

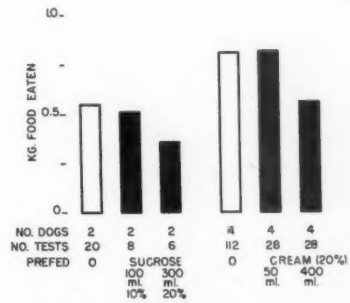

FIG. 3. Effect of prefeeding sucrose solution or cream immediately before offering regular food to dogs. (Data from: JANOWITZ, H. D. and GROSSMAN, M. I. *Am. J. Physiol.*, 164: 182, 1951.[3])

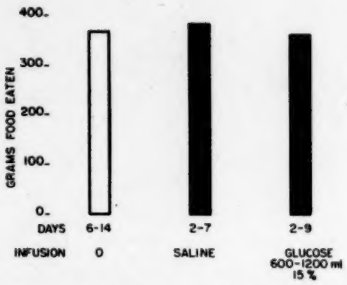

FIG. 4. Effect of intravenous infusion of glucose solutions on oral food intake in dogs. Mean values for five dogs. Number of days refers to number of consecutive days infusions were continued. (Data from: JANOWITZ, H. D., HANSON, M. E. and GROSSMAN, M. I. *Am. J. Physiol.*, 156: 87, 1949.[5])

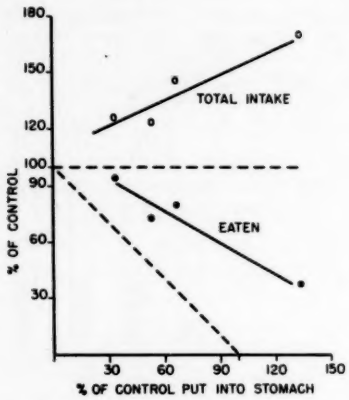

FIG. 5. Effect of various levels of intragastric feeding on oral food intake. Mean values for four dogs for the first two weeks of intragastric feeding. Sloping dotted line is the expected oral intake if oral plus intragastric feeding were to equal the control value. (Data from: SHARE, I., MARTYNIUK, E. and GROSSMAN, M. I. *Am. J. Physiol.*, 169: 229, 1952.[2])

while these nutrients are being absorbed from the gut. It might reasonably be anticipated that these elevations would play a role in regulation of food intake. A direct test of this hypothesis is the study of the effect of intravenous infusion of nutrients on food intake. Glucose is the only nutrient which has been extensively studied in this way. Figure 4 summarizes a portion of the data from a study by Janowitz et al.[5] Intravenous administration of large amounts of glucose immediately before feeding did not depress food intake. Similar negative results were

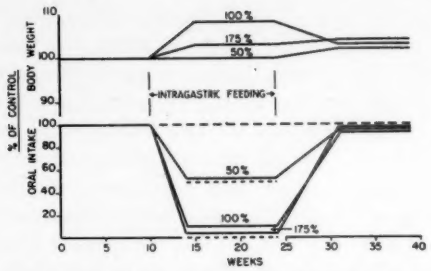

FIG. 6. Body weight and oral food intake of dogs during prolonged intragastric feeding at various levels. Percentage numbers refer to level of intragastric feeding as per cent of control oral intake. Dotted lines indicate expected oral intake if exact compensation for intragastric feeding occurs. Mean values for three dogs. (Data from: JANOWITZ, H. D. and HOLLANDER, F. *Ann. New York Acad. Sc.*, 63: 56, 1955.[7])

obtained by Bernstein and Grossman[6] in studies with both intravenous and intragastric administration of glucose to human subjects. Other aspects of the glucostatic theory of regulation of food intake are considered by other participants of this symposium. Our observations do not support the theory, nor do they disprove it.

THE POST-ABSORPTIVE STATE

The final step in the fractionation of the feeding process is a consideration of the post-absorptive state, the effect of nutrients which have left the digestive tract and the blood. This can be conveniently studied by intragastric feeding performed many hours before oral feeding is measured. Figure 5 is derived from data published by Share et al.[2] During the first two weeks of daily intragastric feeding the depression of oral feeding was not fully compensatory; the sum of intragastric and oral feedings far exceeded the control oral intake which presumably represents the requirement for energy balance. More prolonged studies of this type were performed by Janowitz and Hollander[7] and are summarized schematically in Figure 6. During the initial four weeks of intragastric feeding oral intake progressively decreased until it reached the expected level and thereafter remained at that level while intragastric feeding was continued for ten more weeks. With cessation of intragastric feeding the length

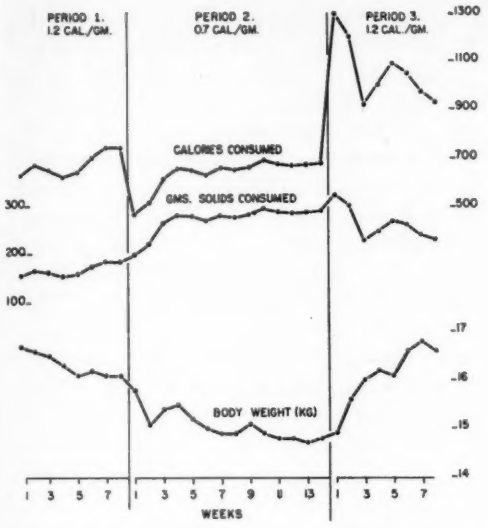

FIG. 7. Effect of change in caloric density on oral food intake and body weight. Mean value for six dogs. During period 2 the regular dog food was diluted with hydrated cellulose. (Data from: JANOWITZ, H. D. and GROSSMAN, M. I. *Am. J. Physiol.*, 158: 184, 1949.[1])

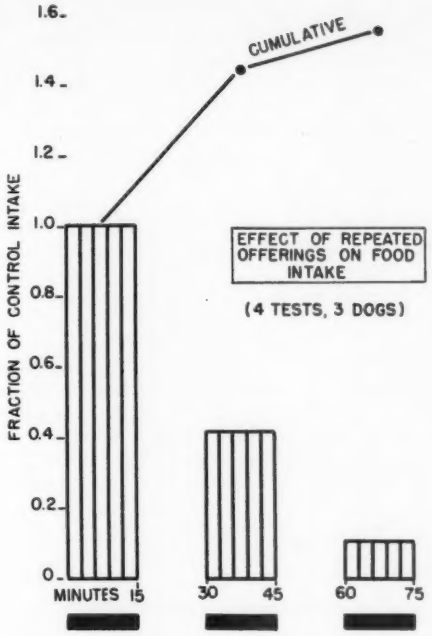

FIG. 8. Food intake during three successive fifteen minute offerings separated by fifteen minute periods of removal of food. The black bars designate periods when food was offered. Comparison is with control period when food was offered once daily for forty-five minutes. (Data from: SHARE, I. and GROSSMAN, M. I. Unpublished data.[10])

of time required for equilibration to be re-established to the original level of intake was even longer, an average of seven weeks. During these periods of equilibration at the onset and offset of intragastric feeding the dogs received large excesses of calories. In the dogs receiving 175 per cent of control intake intragastrically, this caloric excess occurred throughout the intragastric feeding period as well. And yet, *mirabile dictu*, increases in body weight were barely detectable. This is similar to the observation of Fenton et al.[8] who found that certain strains of mice did gain weight when they consumed extra calories as a result of being placed on a high fat diet. When positive energy loads are induced in dogs by intragastric feeding, a long period of time (weeks) is required before equilibration of oral intake occurs.

Variation of the caloric density of the diet produces an analagous situation with negative energy loads. Figure 7 summarizes data reported by Janowitz and Grossman.[9] After equilibration on a regular diet, dogs were fed food diluted with cellulose. Again it will be noted that equilibration at the start and end of feeding of the diluted diet required many weeks.

From the observations thus far presented the following working hypothesis can be derived. Food is metered in the mouth, pharynx and stomach. The nature of the detectors in these areas is not known, but volume appears to be of great importance and hence distention receptors can be postulated. When the signals to the brain from these detectors reach a certain level feeding reflexes are inhibited; eating ceases. The setting of this mechanism is probably determined by energy balance. This setting displays considerable inertia; the volume of food ingested tends to remain constant and only changes slowly in response to positive or negative energy loads. How the energy loads influence the setting, how the tissues tell the brain

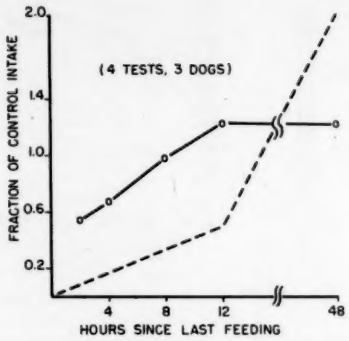

FIG. 9. Effect of interval since last feeding on amount of food eaten. Comparison is with control intake on a twenty-four hour schedule of feeding. Dotted line represents expected intake if amount eaten is directly proportional to length of abstinence from food. (Data from: SHARE, I. and GROSSMAN, M. I. Unpublished data.[10])

how much energy they have stored, is unknown.

PRECISION OF REGULATION

Dogs maintained under standardized conditions of feeding show only small fluctuations in food intake and maintain their body weight with remarkable precision. For example, the data of Janowitz and Hollander[7] show that for three dogs during three ten-week control periods the mean coefficient of variation of food intake was 12 per cent and of body weight 1 per cent. However, small changes in feeding schedule can cause large changes in intake. Some previously unpublished observations made by Share and Grossman[10] illustrate this. Dogs, whose control intake had become stabilized when they were offered food once daily for forty-five minutes, were presented with food three times (for fifteen minutes each time) with fifteen minute intervals between offerings (Fig. 8). The intake during the first fifteen minute offering was equal to the control intake, but food was consumed at each of the two subsequent offerings so that the total intake was in considerable excess of the control intake. Under these circumstances it would appear that fully repleted dogs eat simply in response to being offered food.

Another demonstration of the same type of phenomenon is depicted in Figure 9. Dogs accustomed to being fed every twenty-four hours were fed at longer or shorter intervals. At two hours after the last offering, intake was 55 per cent of control; at three hours, it was 67 per cent; at eight hours, it was 98 per cent; at twelve hours, it was 103 per cent; and at forty-eight hours, it was 101 per cent. It is reasonable to assume that the magnitude of the energy deficit is approximately a simple linear function of time since the last feeding. It is then clear that under these conditions of sudden alteration of feeding schedules food intake is not accurately adjusted to size of deficit. Since these inaccuracies of regulation can be induced even when all feeding is by the normal route, it is not surprising that fractionation of feeding events may fail to produce predicted alterations in intake. It is for this reason that negative experiments cannot rule out hypothetical mechanisms.

It appears that the assumption that regulation is precise, that intake closely approximates deficit, may be invalid for many experimental situations. It follows that deductions based on this assumption may be misleading.

COMMENTS

A number of factors which might be expected to serve as signals for cessation of eating have been considered. I have dealt mainly with those aspects of the problem which my colleagues and I have studied. Additional factors, such as osmotic effects, temperature effects, and others, which may be of as great importance as those studied, have been neglected. This serves to emphasize that the regulation of food intake is a multi-factorial process.

The best working hypothesis available is that these factors operate through the areas of the brain, especially the hypothalamic portions, which are concerned with facilitation and inhibition of feeding reflexes. This aspect of the problem has not been considered because in no instance is it known how a given factor sends its signals to the brain.

SUMMARY

Many factors are concerned in the production of satiety or cessation of eating. Among these are a stomach distended either by food or

non-nutritious substances and, over a relatively long period of time, energy balance. On the other hand, food that has left the stomach, secretion of enterogastrone, or large amounts of intravenous glucose appear to have no effects on satiety. Although it has been postulated that the inhibition of feeding reflexes are carried through the hypothalamic portion of the brain, neither the precise pathways that mediate satiety nor the interrelation of the many factors causing cessation of eating are known.

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DISCUSSION

Dr. John Brobeck (*Philadelphia, Pennsylvania*): These two possibilities you have given us are not mutually exclusive. You said feeding is not regulated precisely because it does not follow energy deficit It is possible that feeding does not follow energy deficit, but it is regulated on some other variable.

Dr. Grossman: I think the fact is that in spite of many types of variation in schedule, activity and so on, regulation is achieved, but the lags are so great that tremendous imprecisions can occur while the equilibration is occurring.

Dr. Brobeck: Regulation is not necessarily directed toward replenishing deficit of energy. Sometimes energy is replenished and sometimes it is not, depending on other circumstances.

Dr. Grossman: I think that statement is also correct. When re-equilibration has taken place, it does not necessarily mean that you have simply repaired deficits.

Dr. Eliot Stellar (*Philadelphia, Pennsylvania*): One may encounter failures of regulation in experiments of this sort due to a number of factors that might be operating. One of them is the factor of habit, which may have entered into the experiments of Dr. Grossman.

In experiments in the related field of specific hungers, a failure to make beneficial selections may occur because of previous habits of ingestion which have led to selection on, say, a taste basis. For example in the experiments of Dr. Harris with vitamin B_1 selection, the animals erroneously followed the flavor of anise, which had been associated with the vitamin B_1. On subsequent measurements when the anise was associated with a vitamin B_1 deficient diet the animals switched and ate the deficient diet, although they had continued to be deficient. There are other experiments which suggest the general principle that taste, habit factors, and other, including emotional inhibition or emotional interference, may enter into experiments on regulation over a period of time, to result in an imprecise measurement or an imprecise result.

Dr. Henry D. Janowitz (*New York, New York*): While the discussion has emphasized the imprecision of these regulations, I think we should not lose sight of the fact that when equilibrium was established, even by such circuitous routes as intragastric feeding, the precision of regulation was precise at the time of equilibrium. Thus, energy deficits were closely corrected by appropriate food intake, granting the initial inertia of the system. Deficits of 50 per cent were corrected, although it took several weeks for this equilibrium to be established, in the absence of pharyngeal and gastric cues.

Dr. Brobeck: There is no one necessary fat content for the carcass of an animal. When one speaks of replenishment of an energy deficit, it implies that the animal is going to eat until it obtains a certain concentration of fat. This is not true. One can change the concentration of fat simply by changing the composition of the diet, the environmental temperature, the availability of water, or the endocrine status of the animal. There are many different changes that will alter the concentration of fat.

DR. JAY TEPPERMAN (*Syracuse, New York*): Some years ago Dr. Stevenson did some experiments in which the responsivity of the regulatory mechanisms was made erratic by shifting suddenly from a high carbohydrate diet to a high fat diet, or vice versa. The suggestion was implicit in this that the regulatory mechanisms had become adapted, or accustomed, to regulating with a good deal of precision on one regimen and then when the regimen was drastically altered, the regulatory mechanisms were no longer adequate to the new situation.

Dr. J. A. F. STEVENSON (*London, Ontario, Canada*): I think that the experiments to which Dr. Tepperman is referring may be those that we did with Lundæk.

While there was some evidence of what you say, this was complicated by what appeared to be another factor. On changing, for instance, from a high-fat to a high-carbohydrate diet, we had the impression that the difficulty in adjusting may have been partly gastrointestinal in that the digestion and absorption processes had themselves become unadapted to carbohydrate. This was borne out by studies on the isolated diaphragms of such animals in that the diaphragms of the animals fed the high-fat or non-carbohydrate diet would not take up nearly as much glucose as those from the high-carbohydrate animals. We thought that the same thing might be occurring in the gastrointestinal tract.

I think that while there was an apparent upset of regulation, these experiments also showed this other factor, namely, an effect on the local tissues involved in the handling of food by the body.

Appetite in Man

WALTER W. HAMBURGER, M.D.*

IT IS A pleasure for me to join with investigators of varied disciplines to exchange experience and views on the regulation of food intake as part of the larger area of energy balance. The fact that so many disciplines are represented in this symposium attests to the growing awareness of how multifaceted and complex are these subjects. In this paper I am concerned with regulation of food intake, and will define my area of training and interest, the material at my disposal and the method I use in trying to understand the regulation of food intake.

MATERIAL AND METHODS

My material has been confined to the human animal; as a physician, I study people who are ill. I have focused my attention on the nutritional problems of my patients for the past decade, since my first psychiatric study of obese patients.[1] Since that study I have been alert to and kept notes on the symptoms of anorexia, food habits, hypophagia, hyperphagia and changes in weight in the patients I see in the practice and teaching of medicine. These patients are the men and women in the Strong Memorial or Rochester Municipal Hospitals as in- or out-patients, division or private status, usually in the Medical and Psychiatric Services and occasionally in Surgery, Obstetrics-Gynecology or Pediatrics. I have studied unselected groups of obese patients in the Medical Clinic and unselected groups of depressed patients in our psychiatric wards. I have also studied patients in a select group referred for psychiatric and psychoanalytic consultation or treatment. Some of these patients have had anorexia nervosa, some were obese and a few had bulimia. Many have been ill with emotional depressions which usually involved disturbances in food intake and body weight. The obese patients in the unselected group at the Medical Clinic were not necessarily there primarily because of their obesity, but had heart disease, arthritis, hypertension.

My material, then, is the traditional material of the clinical investigator—human, adult and ill. The sources of primary data were the following:

I. Obesity
   - A. Eighteen selected patients referred to psychiatry[1]
   - B. Unselected patients from Medical Out-Patient Department
   - C. Individual patients seen in consultation and psychotherapy
   - D. Two patients undergoing psychoanalytic therapy

II. Emotional depressions
   - A. Individual patients seen in consultation, psychotherapy and psychoanalytic practice
   - B. Review of one hundred unselected in-patient records[7]

III. Anorexia nervosa
   - A. Individual patients seen in consultation and psychotherapy
   - B. One patient undergoing psychoanalytic therapy[5]

IV. Bulimia
   - A. Individual patients seen in consultation and psychotherapy
   - B. One patient undergoing psychoanalytic therapy

V. Dreams of food and eating
   - A. Retrospective studies of 320 dreams in five psychoanalytic cases[4,5]
   - B. Predictive studies in process in five psychoanalytic cases

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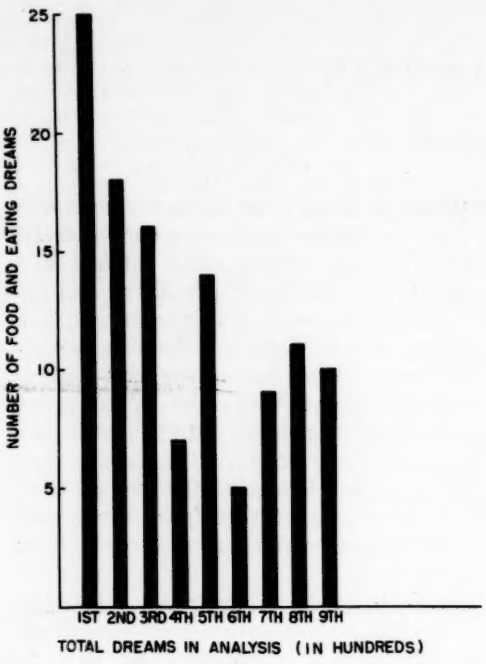

FIG. 1. Case 1. The incidence of food and eating dreams in relation to total dreams in analysis.

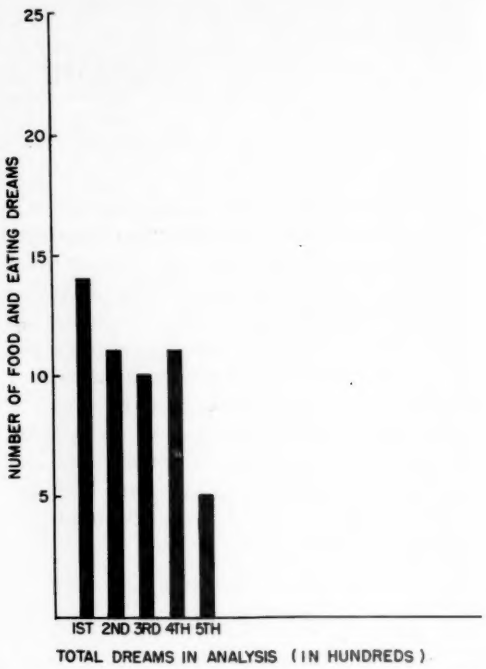

FIG. 2. Case 2. The incidence of food and eating dreams in relation to total dreams in analysis.

My methods of study have been exclusively psychologic because my training has been as a psychiatrist and psychoanalyst. I can only read about the physiologic and metabolic regulations of food intake as studied by investigators specializing in those technics.

Psychologic methods used were the psychiatric interview, a Food and Eating Sentence Completion Test and the psychoanalytic study of the repetitive dreams of food and eating reported by patients during psychoanalytic therapy.

TABLE I
Numerical Summary of Dreams of Five Patients

Patient No.	Total No. of Dreams	No. of Dreams of Food and Eating	Percentage of Dreams of Food and Eating
1	900	115	12.6
2	571	57	9.9
3	341	36	10.6
4	116	21	18.1
5	491	91	18.5

The psychiatric interviews ranged from a single hour's discussion with an unselected patient from the Medical Clinic to 389-hour interviews with an obese patient in psychotherapy. Some of these interviews have been taped, transcribed and then summarized in an Obesity Questionnaire to provide some objective data which can be reviewed by me and/or other "judges."

I devised the Food and Eating Sentence Completion Test in the hope of developing an instrument for studying "oral urges" in particular and the interplay of food habits with personality in general, in a more systematic and shorter period of time than is permitted in psychiatric interviews. This test is being explored by two colleagues in our department (Vivian Harway, PH.D. and Howard Axelrod, PH.D., M.D.). We do not yet know how discriminating or useful this instrument will be. It can be easily administered because the pa-

tient-subject records his own responses. In this way, groups of subjects can take the test together. The use of this instrument with medical and college students is the only time any of my methods have been employed with healthy people.

Dreams concerned in their manifest content with food and eating symbols have been spontaneously reported by all of my patients under psychoanalysis whether or not they had nutritional or gastrointestinal symptoms. These dreams appear to reflect oral urges in the mind of the dreamer. Being repetitive, these dreams permit serial study and some quantitation (Table 1, Figs. 1, 2). Such oral urges during sleep can and must be distinguished from the eating behavior of these patients during their waking hours. I regard these themes of food and eating in dreams as a representation, usually in visual symbols, of the person's appetite at the time of the dreams. The latent (unconscious) meaning of these symbols can be determined during the waking state by the psychoanalytic technic of free association. Only then can the observer and the dreamer discover whether the symbols in the dream referred to the dreamer's nutritional appetite or to other appetites. The spontaneous associations to these food and eating symbols usually disclosed non-nutritional appetites, i.e., cravings or wishes for dependent, interpersonal gratification, for sexual satisfaction and for the expression of hostilities. (For an excellent discussion of such an approach to symbols of food and eating in dreams, see Kubie.[2,3])

This method of clinical investigation allows the observer to study the subjective aspects of human appetites, inasmuch as the dreamer can report his thoughts and feelings associated with the food and eating symbols in his dreams. This is obviously a technic applicable only by human observers to human subjects. The technic is that of free association in the context of psychoanalysis. The experimental "instrument" is an investigator thoroughly trained in psychoanalysis.

Thus far, I have studied 320 food and eating dreams in the course of the psychoanalyses of five female patients (Table 1).[4,5] Note that the highest mean incidence of these repetitive dreams was in a patient (5) who had anorexia nervosa. One patient (4) with a similar high incidence of these "appetite dreams" had bulimia. Another patient (3) was obese. Two patients (1, 2) were of normal weight and had no gastrointestinal or nutritional symptomatology. Note, however, the higher percentage of these food and eating dreams in one of the normal patients (1). I interpret this as reflecting stronger "oral urges" in this woman than in the second patient (2). Additional data to support this interpretation are available from a comparison of other material from the two analyses.

The first five cases were studied retrospectively from my notes after the termination of psychoanalysis.[4,5] Similar predictive studies of the occurrence of these food and eating dreams are in process with four of my own patients currently undergoing psychoanalysis. As a control study, I have made predictions in a fifth patient being treated by another analyst.

An unexpected finding, perhaps more germane to the discipline of psychoanalysis than to this nutritional symposium, was that the incidence of the reported food and eating dreams decreased as each individual analysis progressed (Figs. 1, 2). I interpret this as reflecting a change in each patient's "oral urges" as she progressed in psycho-sexual maturity during the analysis.

Such dream studies, in their natural psychoanalytic context, also shed light on the psychologic and symbolic meanings of specific foods, tastes and aversions, in addition to the dreamers' appetitive urges. In my experience, this is potentially useful in the further investigation of food preferences, idiosyncrasies and food allergies, certain hysterical conversion symptoms and various psychosomatic disturbances involving function of the upper gastrointestinal tract. For example, one patient (3), who was obese, referred to in Table 1, had a diagnosis of an allergy to eggs (with symptoms of nausea and vomiting). After discovering during analysis the relation of five dreams of eggs with her childhood notions of oral impregnation, her "allergic" reaction to eggs disappeared.

These dream studies, apparently reflecting human oral urges unaccompanied by eating behaviors, emphasize the need to distinguish clearly between the two. In the past, we have confused oral urges with eating behaviors, hunger with appetite and all with body weight. Such an error is not unexpected if the human observer is studying only infra-human animal material. In such subjects only behavioral manifestations of eating or not eating and weight changes are observable. Fortunately human material permits an introspective report from the subject which can be compared and contrasted with behavioral observations of one investigator or multiple "judges." An excellent example is Stunkard's paper on the dissimilar subjective evaluations of "hunger" by obese and non-obese women, in marked contrast to his observations of their similar gastric patterns of peristaltic activity.[6]

CLINICAL OBSERVATIONS AND INFERENCES

The following is a summary of my observations and the inferences I have drawn from my clinical and investigative experiences during the last ten years. More detailed data with illustrations from cases may be found in other publications.[1,4,5,7–10]

(1) Patients with hyperphagic and hypophagic symptoms usually have been over- and undereating in relation to emotional needs not otherwise satisfied at the time. The data for this come from the medical history of the patients and subjective reports of their altered eating habits in relation to events in their life. The apparent nutritional symptoms appeared to be a method of dealing with and yet avoiding underlying emotional problems. A corollary discovery was that most of these patients did not evince gross abnormalities in metabolic, feeding or satiety regulations of food intake. This was only by current clinical examinations commonly performed in their medical examination: thorough physical and neurological examinations, roentgenograms of the skull, electroencephalograms, basal metabolic rate, uptake of radioactive iodine 17-ketosteroids, glucose tolerance tests, etc. Many obese patients, severely and chronically overweight, did have abnormal glucose tolerance test results and/or were known to have diabetes. Many did not have demonstrable hyperphagia. Therefore, metabolic derangements appeared probable. However, it will take more refined clinical tests to establish such pathology with greater definition. Our present methods of establishing or eliminating the existence of hyperphagia and hypophagia, i.e., accurate caloric intake, leaves much to be desired, especially with out-patients. I am convinced that many patients are genuinely unaware of how much they eat. They often revise their estimates on subsequent contacts with the same interviewer.

The development of symptoms of over- and undereating in relation to underlying emotional conflicts is understandable in terms of *substitutive adaptive mechanisms*. Eating behaviors are substituted for other non-nutritional needs of which a given patient may or may not be aware. In psychiatry we regard such substitutive devices as *ego defense mechanisms*. These mechanisms permit a person to deal with and yet avoid difficult emotional problems.[11] Corroboration of this conception can be found in the hypnotic experiments of Seitz[12] in which the symptom of hyperphagia in an obese woman was deliberately replaced by a variety of substituted symptoms.

In ethologic terminology, this concept is an example of displacement activity.[13] It is of interest that Tinbergen and other ethologists previously referred to displacement activities as "substitute activities." My patients, who have utilized hyperphagia and hypophagia as a substitute activity, have apparently done so for the same reasons given by Tinbergen in explaining displacement activities of infra-human organisms, i.e., displacement from the conflict of two antagonistic drives: a strongly motivated (internal) drive with lack of external stimuli for the release of the consummatory acts belonging to that drive. In my experience with human beings, one or both of the antagonistic drives are not apparent to the person (repressed) and hence lead to the displacement activities of hyperphagia or hypophagia by unconscious defense mechanisms. Lack of external objects for satisfaction of drives also is influential.

(2) The use of the responses of eating and not eating as substitute activities for unexpressed emotional needs ranges from occasional to frequent to habitual. Many healthy people eat less during a period of grief and more during a happy period. However, certain people frequently utilize such eating behaviors in reaction to many moods and many stresses. Such hyperphagic responses may lead to what Bruch termed "reactive obesity."[14] Similarly, I have studied a great many food addicts who are compulsively habituated to hyperphagia regardless of transient moods or life situations. I have attempted to classify such emotionally determined patterns of hyperphagia in previous publications.[1,8] I felt the need to make such a trial classification because, within the parameter of psychologic regulations of food intake alone, there are major individual differences in psychopathology, prognosis and, therefore, rational recommendations for treatment. Similarly, emotionally determined hypophagia ranges from occasional to habitual. Habitual hypophagia is seen in severe cases of anorexia nervosa, psychotic depression and catatonic schizophrenia when slow self-destruction is brought about by hypophagia leading to inanition, cachexia and, all too frequently, death. In these malignant cases I have had the impression that concomitant or secondary metabolic factors are influential, but we still have difficulty in specifying them.

I believe that the term "compulsive" for those patients who habitually over- and undereat is technically (psychiatrically) quite appropriate and not a misuse of terms. A compulsion is a symptomatic behavior pattern which a person uses repeatedly as a substitute for some other drive discharge or satisfaction. It is just because the symptoms of eating or not eating have already been substituted for a primary non-nutritional drive, that satiety is never achieved and the compulsion continues. Then we clinicians complicate matters further by trying to treat the presenting nutritional symptom of hyperphagia or hypophagia by nutritionally logical means, only to often fail because neither we nor the patient recognize the underlying primary conflict or drive need.

(3) The relation between hyperphagia and hypophagia is much closer than is generally appreciated. The same person may be hyperphagic at one time in his life and hypophagic in different circumstances. Patients with bulimia often have a base line hypophagia, with sprees of hyperphagia. One patient (4) in my first study of dreams of food and eating had anorexia nervosa at the age of fifteen, occasional sprees of hyperphagia developed, and by the age of seventeen had well defined bulimia with weight gains of fifty pounds in eight weeks, which made her "reactively" obese.[4] Meyer[15] reports a similar case relating the similarities of obesity and anorexia nervosa. Thorough medical histories of obese adults often reveal normal weights or thinness in childhood or in adulthood, prior to significant changes in events in the patients' life. Conversely, if we follow the histories of patients with anorexia nervosa long enough in remission, we learn that a certain number become obese. Kenyon states that anorexia nervosa may develop in obese persons after dieting.[16] I believe we can learn a lot from those patients who convert from one symptom and syndrome to the other. I believe we can learn the most about etiology, the subtle interplay of pathophysiology and psychopathology, in acute cases and from those patients with only a minor problem hyper- or hypophagia, of overweight or underweight. The severe chronic patients have so many secondary and tertiary complications that the investigative yield is limited. Also, the prognosis is so poor and the patients so ill that therapeutic needs may become too demanding for the clinical investigator. Conversely, the therapeutic rewards in the successful treatment of the lesser nutritional disturbances are great and add retrospectively to our etiologic hypotheses. An example is a woman with anorexia nervosa who regained twenty-three pounds as well as her menstrual periods after four and one-half years of amenorrhea.

(4) What feelings, drives or conflicts have my patients been dealing with by their substitutive nutritional behaviors? I have learned that they may run the gamut of human feelings and conflicts. However, the most common finding has been feelings of depression

(boredom, loneliness, sorrow, blueness, helplessness, guilt, self-destructiveness, self-hate and hopelessness). In a sense, anorexia, the loss of desire to eat, is as much a depressive feeling as the affects just listed.[17]

In this affectual spectrum, hypophagia is the behavioral symptom expressive of a serious degree of depression. The constellation of anorexia, hypophagia and weight loss constitute some of the "biologic signs" of psychotic (serious) depression and have been so regarded for many years in clinical psychiatry. My own clinical experience has convinced me that anorexia and hypophagia pivot around the person's feelings of guilt. These patients believe they are too unworthy to "deserve" the pleasure of eating (and consequent weight gain and health). This does not imply that metabolic factors do not contribute to anorexia and hypophagia. On the other hand, less serious degrees of loneliness and sadness are often dealt with by hyperphagia. Indirect evidence for this is the frequent occurrence of emotional depressive feelings when obese patients successfully curb their hyperphagia by diet and/or drugs.[9,18] Some healthy subjects subjected to voluntary semistarvation, also became emotionally depressed.[19]

In relation to concepts of separation and loss developed by colleagues in my department,[20–22] it has been interesting to note how frequently the symptoms of anorexia and hypophagia as well as hyperrexia and hyperphagia have been closely preceded in time by such experiences. It appears that real or phantisied separation or loss may contribute to the psychopathology of appetite which in turn leads to altered food intake and then, according to various predispositions, neurotic or psychotic depression, reactive obesity or reactive states of inanition. Bruch quotes Hume's "A Letter to a Physician" (1734) in which he tells how he suffered a "severe mental depression" when the publication of his new philosophy had not achieved immediate recognition. Following this loss of self-esteem, he developed a ravenous appetite and changed from a lean to a "sturdy, robust fellow."

The reaction of hyperphagia to separation or loss has a psychologic logic to it. This can be expressed as self-feeding (an auto-erotic behavior), an attempt to substitute or replace the lost person, or the loss of an ideal or self-esteem, as in the instance of Hume. Such a substitutive attempt is not confined to food. A child separated from its mother may suck its finger for solace. Some people react to a loss by turning to alcohol, drugs or excessive smoking. The person who is predisposed to turn to food reacts with hyperphagia. A man with high plasma pepsinogen might conceivably react to such a loss by the formation of peptic ulcer.[23,24] The increase in oral urges appears to be the common factor in these different reactions, and these must be studied apart from the clinical syndromes.

Psychoanalytic studies of dreams of food and eating appear to provide such an opportunity to study oral urges apart from their behavioral manifestations. There is an increase in the reporting of such dreams during and following separations and/or losses. The food and eating symbols reflect a regressive wish for security, succorance and dependent gratifications. These dream symbols in other phases of analysis, are regressive substitutes for sexual gratification (oral-erotic pleasures rather than genital-erotic) or for aggressive release (through dream symbols of biting, chewing and swallowing).

(5) From listening to my patients tell of their food habits and, especially, of their dreams of food and eating, I have learned something of the psychologic significance of specific foods to specific persons. A few symbols occur with frequency, e.g., milk is often a symbol of a person's relationship to his mother. This is hardly surprising when we reflect on the nature of our children's first feedings. Meat is usually a symbol of masculinity and strength. Eggs symbolize impregnation to many women and are usually connected with confusion in childhood of the theme of oral impregnation.

I have also learned that food textures are important in their mental representation, i.e., many sick people prefer the bland, soft cereals, soups, jello and custard that they enjoyed as children. In aggressive angry moods, some people prefer meat, coarse vegetables,

raw carrots or celery; food that they can "get their teeth into." Foods associated with particular cultural backgrounds, socio-economic status and religious rituals are well known: Thanksgiving turkey, English mutton, Russian caviar, spaghetti, chopped liver, holy bread and wine, etc.[2–5,10,25–27]

(6) Clinical observations have led me to appreciate the distinctions between oral urges and eating behaviors, and both from body weight. The disease states of obesity or inanition are multidetermined syndromes. There must always be varying elements of disturbance in physiologic and psychologic parameters in each case. The final syndromes only resemble each other superficially and we need more precise methods of multifactorial diagnosis. There is no doubt that in some cases, hyperphagia may not be present, and a metabolic derangement in utilization and/or energy expenditure leads to deposition of excessive fat. Just as man is indivisible in his integrated biologic construction, each of these syndromes is a psychosomatic disease in which pathophysiology and psychopathology are interdependent. This integrated concept is well developed by Mirsky, using the multi-determined syndrome of peptic ulcer as his model.[28] Bruch[14] expresses a similar formulation, concerning the pathogenesis of obesity, in her book. She states that "In each case of obesity we are dealing with at least three elements: the constitutional endowment, the conditioning psychodynamic life experiences, and the precipitating traumatic events."

Just as Mirsky has worked out high plasma pepsinogen as one (biochemical) predisposition to peptic ulceration, I am exploring the hypothesis that the incidence of dreams of food and eating may correlate with a (psychologic) predisposition to certain nutritional or gastrointestinal diseases, including peptic ulcer. Currently, I am studying the statistical aspects of these repetitive dreams in the hope that even crude quantitation of "orality" will permit some clinical predictions. This is a purely psychologic approach to oral urges and influences the psychologic regulations of food intake. I consider this parameter of nutritional regulations to be subsumed under appetite, as contrasted with regulations of hunger. I believe we can make greater progress with our investigative and clinical work if the conceptual differences between hunger and appetite are clarified.

TABLE II
Concepts of Hunger and Appetite

Hunger	Appetite
Unpleasant apperception	Pleasant apperception
Motivates aim of relief	Motivates aim of gratification
Apperception of the present	Present, past and future
Species specific	Species specific
Mechanisms resemble infra-human forms	Mechanisms unique to man
Present at birth (modified by experience)	Conditioned and learned (partly innate taste)
Periodic ("biologic clock")	Irregular
As an instinct:	As a drive:
A physiological concept	A psychological concept
Involuntary	Purposeful
Imperative	Elective
Immutable, stereotyped	Permits of delay and distortion
Unconscious	Conscious
Only food gives satiety	Food substitutes "satisfy"

HUNGER AND APPETITE

The following concepts of hunger and appetite stem from clinical observations discussed in the preceding section. These are summarized in Table II.

Hunger may be regarded as a reflection of metabolic needs of tissues, that is, the need for nutritional replenishment. I presume this is fundamentally a biochemical reaction which begins at the molecular and cellular level. However, we know that such metabolic needs are reflected in and regulated by a host of enzymatic, hormonal, vascular, gastric, hypothalamic and other functions of the central and autonomic nervous systems. I will limit my definition of the hunger state to a disturbance in these biochemical and physiologic regulators of nutritional homeostasis. The term "hunger" is used herein only as a referent to biochemical-physiologic processes.

Hunger regulations may be regarded as species-specific, genetically determined, present

at birth and, therefore, instinctive in the sense that Tinbergen and Thorpe use the term.[29,30] Some (gastric) evidence that hunger mechanisms are innate was furnished by Carlson in 1912.[31] He demonstrated that newborn babies exhibit " . . . the typical periods of tonus and hunger contractions of the adult, the only difference being the greater frequency of these periods in the young."

To borrow terminology from the ethologists, I would suggest that the complex reflexes of sucking, swallowing ("eating behaviors") of human neonates represent the "consummatory acts" of innate, instinctive hunger patterns. Such nutritional behaviors partake of all the characteristics of instinct summarized by Thorpe[30]: "a) It is an inherited system of coordination; b) it involves more or less rigid inherited action patterns; and c) more or less rigid inherited releasing mechanisms." When we label such feeding behavior as expressions of "hunger" we are inferring a causal connection which, although it might be valid, is an instance of "adultomorphizing" our material. I am in complete accord with Tinbergen[30] when, using a similar example of a food-seeking animal, he writes, "But when the conclusion that the animal hunts because it is hungry is taken literally, as a *causal explanation*, and when it is claimed that the subjective phenomenon of hunger is one of the causes of food-seeking behavior, physiological and psychological thinking are confused . . . . Hunger, like anger, fear and so forth, is a phenomenon that can be known only be introspection. When applied to another subject, especially one belonging to another species, it is merely a guess about the possible nature of the animal's subjective state." Tinbergen thus expresses the strict scientific viewpoint. Why have we psychologists and physiologists been so loose in our use of the terms "hunger" and "appetite" for such a long time? I think it is because we, as human observers, with our human capacity for introspection, having all felt the (subjective) pangs of hunger, have assumed that he who eats is hungry.

The molecular level of nutritional homeostasis is a biochemical affair which, as far as we know, has no direct mental representation and remains unconscious. However, objective experience with an unfed crying neonate and introspective experience of unfed human adults suggest that when certain thresholds levels are exceeded, the need for nutritional replenishment is represented mentally. Attempting to be objective about the infant, (or other infra-human animals) we term their behaviors the "hunger state." For the complex of subjective sensations, feelings and perceptions in ourselves, we apply the term "hunger." These feelings are subjective, known only by introspection and hence the term "hunger" is a purely psychologic construct. I find it confusing to use the term hunger for a complex of subjective perceptions when basically we are referring to metabolic, biochemical and physiologic processes.

To be less ambiguous, I therefore propose that we restrict the term hunger to the physiologic regulations and processes and apply the term *appetite* to the psychologic regulations and concepts.

The mechanisms by which nutrient needs at the cellular level are conveyed to the central nervous system, permitting the conscious apperception we call hunger, are just being elucidated. Due to the investigations of Cannon, Washburn and Carlson, we know most about the correlation between the "pangs of hunger" and gastric peristaltic activity. More recent work involves the mediators of the autonomic nervous system, the hypothalamus and its possible chemo receptors, the limbic system and hormonal and enzyme "messengers."

By the term hunger, biochemists are referring to molecular, metabolic, enzymatic and cellular processes; physiologists to gastric and hypothalamic organ functions; ethologists to certain food-seeking and eating behaviors; and the psychologists to a cluster of subjective sensations and perceptions. This confusion should neither surprise nor overwhelm us; in all biologic adaptations there are multiple levels of regulations and patterns of processes ranging from the molecular to the molar, a hierarchy of individual and yet interrelated processes.[31–35]

Where and how do these unconscious nutri-

tional regulations from cellular, hormonal and organ levels become conscious apperceptions of the need for food and the desire to eat? I suggest that this takes place in the highest integrative levels of the human nervous system, namely in the limbic and neocortical system. When a healthy human adult feels "hungry" and purposefully tries to find food and eat it, he is expressing nutritional regulations which combine in one complex integration all the manifold physiologic and psychologic mechanisms involved in the regulation of food intake.

Evidence to support the concept that the cerebral cortical system acts as an integrator in nutritional regulations is threefold: (1) clinical observations of cerebral cortical disease; (2) studies of nutritional regulations of human infants with incompletely developed cerebral cortices; (3) experimental stimulation and destruction of the limbic and neo-cortical systems in animals.

It has long been known that strokes, epilepsy, encephalitis, tumors and degenerative disease of the cortex may be accompanied by or followed by an unusual increase in hunger, oral behaviors, bulimia and changes in gastric secretory and motor functions, blood sugar, and the like. These are often single case reports like that of Watts[36] of a patient with a frontal tumor who exhibited "morbid hunger." In reviewing such clinical observations in relation to disease of the precentral motor cortex, Aring[37] describes the oral behaviors of reflex sucking, mastication, licking and swallowing. He also discusses the limitations of neuro-anatomic localization in such mixed clinical pictures. Observation of human postlobotomy and topectomy provides more precise information.[38–40] These data show definite trends toward an increase in gastric and small intestinal motility, an increase in food intake and an irregular increase in weight (15 to 25 pounds). Trauma to the cortex may also produce nutritional abnormalities. I recently saw a ten year old boy who had bulimia (and left-sided hemiparesis) and continuously thought and talked about food, following a depressed skull fracture of the right temporo-parietal region. Stunkard studied a nineteen year old man, who was comatose with a cerebral cortical injury following an automobile accident. Using the gastric balloon technic, he discovered that the man had excessive gastric peristaltic activity of the "hunger contractions" type[41a] The patient also exhibited sucking and other involuntary oral behaviors. In response to various test procedures, this patient would bare or grind his teeth and display affects which Stunkard described as sham rage. The reader gains the impression of a "decorticated preparation" with release of lower centers from higher center control.

Many of the aforementioned clinical pictures accompanying cortical disease, injury and operation remind me of the behavioral pictures of the human neonate and young infant. Morphologically and functionally, these subjects, too, have incomplete cerebral cortical control of somatic, visceral and psychologic processes, including nutritional regulation. Sucking, mouthing, biting behavior appears to the adult observer to be involuntary, random or "reflex" in character. It is most intriguing to realize that Carlson's description of the gastric activity pattern of human neonates[31] is quite similar to Stunkard's patient, who has been described. Both situations may be expressing motility patterns of the stomach in the absence of mature and healthy cortical regulatory centers.

The most precise data correlating cerebrocortical localization and nutritional functions are now coming from experiments on stimulation and destruction with infra-human mammals, especially primates. Anand[41b] and Teitelbaum[41c] reviewed this subject at this symposium. I have the impression that progress in this area relates to the study of "higher" mammals, especially monkeys. That is, phylogenetically, these primates have a greater cerebral cortical development and hence the control of "lower" nutritional regulatory centers is more specialized and complex. For example, in their 1955 report, Anand, Dua and Shoenberg[42] report a different behavioral response in "hypothalamic-aphagic" monkeys than in cats with comparable lesions. The monkeys were still capable of biting and swallowing and could be kept alive if the

experimenters put food into their mouths. The authors concluded that hypothalamic feeding reflexes were under greater cerebral control in the monkeys.

Subsequent experiments of Anand and Dua[43] indicated that limbic stimulation produced sniffing, biting and chop-licking behaviors described as "eating automatisms." They noted that the induction of "eating automatisms" by limbic stimulation was not followed by an increase in food intake such as they had found following stimulation of the lateral hypothalamus.[44] The similarity between these oral limbic responses and the oral automatisms of the human patient with disease of the frontal lobe or postconvulsive phenomena is striking. This differential reaction of "eating automatism," following limbic stimulation as contrasted with an increased food intake following hypothalamic stimulation, provides experimental substance for clinical observations in man; mouthing, sucking and oral behaviors are quite distinct from eating behaviors. It is also consistent with the ethologists' behavioral distinction between eating as a "consummatory" act and food-hunting activities termed "appetitive" behaviors.

Confirmation of these experimental data is being gathered by independent investigators.[45–48] MacLean[46,47] emphasized that the limbic stimulation produced marked affective responses accompanying these oral activities. Again it is impressive that MacLean's descriptions are so similar to those of Stunkard,[41a] describing "sham rage" accompanying baring and grinding of the teeth in his patient who was comatose. It was because of such combinations of oral and emotional responses that MacLean postulated that there are limbic centers, involving especially the amygdalar circuit which are "... concerned with emotionally determined functions pertaining to the preservation of the self."[46]

Experimental stereotaxic lesions in the frontal and temporal lobes of cats and monkeys[48] and lesions of the supra-optic nuclei in rats[49] led Anand et al. and Bruce and Kennedy to the same inductive conception that there are two types of urges to eat, one located in the hypothalamus, "hunger," and the other located in the cerebral cortex, "appetite. The cortical influence may modify or even reverse the effect of the hypothalamic factor. These differences are much more marked in monkeys than in cats. Anand et al., attribute this to the (phylogenetically) more marked "encephalization" of the monkey. These authors therefore refer to "primitive hunger" at the hypothalamic level and "discriminating appetite" in the brain.

I am in complete accord with the concepts of these neurophysiologists experimenting with infra-human material in the laboratory. I believe this accord is a reflection of John Hughlings Jackson's remark in 1897 on the "Relations of Different Divisions of the Central Nervous System to One Another and to Parts of the Body: As to the nature of the relation of consciousness, or synonymously, mentation, to activities of the sensori-motor nervous arrangements of the highest level, I have no hypothesis; *I assume concomitance of psychical states with nervous states of at least the highest layers of this level*"[34] (my italics). In other words, when the physiologists and the psychologists converge on the area of cerebral cortical regulations of food intake, I believe we are examining the same nutritional phenomena with different technics and observer-perceptions: the physiologists, the physical aspects in the brain; the psychologists, in its psychologic function as mind. We can consider the possibility that dreams of food and eating represent the same preconscious oral urges (in mental imagery) that limbic stimulation produces as involuntary oral activities (neuromuscular behaviors).

We are now in a position to define appetite as a psychologic expression of mind. As a mental construct, appetite partakes of all aspects of mentation: mnemonic, symbolic representation, perception, affect, cognition, displaceability, etc. I assume that the site of these mental activities we are now calling appetite is in the cerebral cortical system. If we follow McLean's current formulations, and those previously held by Kubie,[2,3] we might think that the affect and drive aspects of appetite are concentrated in the limbic system (visceral brain, involving hypothalamus);

the perceptual, cognitive, symbolic aspects of appetite, in the neocortex.[46] Appetite, as a function of mind, is interposed between the instinctual demands of hunger and the goal-directed acts of food seeking and eating behaviors. As Engel writes, "Man's capability of interposing a delay between the internally arising instinctual force and the final action is the most important determinant of his total psychologic behavior."[50] It is this mental function of the cerebral cortical system of the adult man which permits the apperception we call hunger, memory of the relief of hunger by eating, planning to find food and then purposefully performing the consummatory act of eating. This same mental function permits voluntary dieting or self-starvation as well as equipping man to hunt, fish, cultivate and store food. At less conscious levels there may be involuntary delays, substitutions in this mental faculty which we then view as psychopathologic distortions of appetite which contribute to maladaptive regulations of food intake.

With respect to the regulation of food intake, appetite may be defined as a psychologic desire to eat. However, the dictionary's definition of appetite has no immediate reference to food, eating or nutrition:[51] The first definition of appetite is "An inherent or habitual desire or propensity for some personal gratification, either of body or mind; (a) craving." This ambiguous definition of appetite emphasizes man's psychologic desire for pleasure.

Cannon's views, as a physiologist, correspond with that of philologists. "Behavior may be directed either by movements to get rid of disturbing, annoying stimulation, or by movements to prolong or renew agreeable stimulation. Hunger and thirst belong to the first category . . . On the other hand, experience may condition behavior by revealing that a certain food or drink is the cause of unanticipated delight. An appetite for the repetition of this experience is thus established; the person beset by an appetite is tempted not driven to action—he seeks satisfaction, not, relief."[52]

Thus, Cannon contrasted the pleasurable aspects of appetite with the unpleasant perceptions of the hunger state. He also referred to the experimental, conditioned and learned aspects of the desire to eat (Table II).

Carlson expanded this theme when he wrote, "We may ask whether this gap between the pure sensation of hunger and the ingestion of food in the newborn is not in reality bridged by the factor of appetite . . . Appetite, as we know it, cannot be separated from our memory of past experience with food, that is, the taste, smell, and appearance of food. In fact, it appears to be essentially pleasant memory processes of these past experiences, and the 'urge' in appetite may be only a special case of the general desire for pleasure."[31]

Such concepts make explicit that appetite is a result of experience and memory of eating; not solely a relief from the displeasure of hunger, but an anticipation of the pleasure of eating in the future. Such memory and anticipation require a functioning mind and, in turn, enough healthy development of the cerebral cortex to permit such mental registration and representation..

Carlson also mentions the sensory modalities of taste, smell and visual appearance of food which are associated with food, eating and appetite. The undeveloped brain of the human neonate does not permit some of these sensory associations at birth. There is evidence from infra-human animals, as well as man, that taste is genetically determined.[52,53] This is a neurophysiologic property of the organism which makes it probable that the capacity for taste is present at birth[54] but may require further cortical development for discrimination.[49] In any event, to be consistent in our definitions, if there is an innate, mechanism of taste present at birth we should include it in our biochemical-physiologic parameter of hunger regulations and not as a part of our psychologic construct, appetite. In contrast, we know that human babies are unable to register and comprehend the visual sensation of food until many weeks or months of development of the brain and experience have elapsed. In relation to these various sensory modalities associated with food and eating, it is interesting to note that my adult patients' dreams of food and eating have been almost exclusively in visual sensory representations

Dream tastes or smells of food, sensory modalities presumably developed earlier in the ontogenetic history of the dreamer, are rarely reported. This was also Knapp's independent finding.[55] This suggests that the "choice" of sensory and symbolic imagery in dreams is determined in part by ontogenetic and phylogenetic properties of the human organism. Kubie has discussed the relationship between symbolization and viscerosomatic processes,[2] including in dreams,[3] but his interpretation of the preponderance of visual imagery is different than mine.

The preceding discussion emphasizes our need to view nutritional regulations ontogenetically. It appears that certain subcortical feeding reflexes are operative at birth which permit the neonatal "consummatory acts" of sucking and swallowing. I believe these behaviors should be regarded as hunger regulations which, lacking a mature brain, must be regarded as group reflexes[56] which are largely stereotyped and not always effective. I assume, teleologically, that these reflexes are set into motion by the instinctual biochemical-neurophysiologic regulators of hunger. Only the non-specific cry of the unfed babe permits any psychologizing and then it is entirely our adult (common sense) interpretation that (1) the infant "feels" hungry and (2) that he is crying "to be fed." Thus, there is no evidence for any appetite regulations of food intake being present at birth. It is true that both mothers and nutritional investigators may interpret an infant's motor restlessness, mouthing behaviors, autonomic changes and cry as a state of hunger. The fact is, that newborn babies exhibit such undifferentiated mass reactions to a variety of known and unknown stimuli from within and outside themselves which we adults infer to be "unpleasant" stimuli. Further development of brain and mind is necessary before we can be sure that the infant "feels hungry" and before his full repertoire of taste, smell and visual aspects of appetite sensations is available. It is only with repeated cycles of hunger–eating–satiety, over many months, that the infant learns purposeful, goal-directed, coordinated food-seeking behaviors.

I presume (from the literature on child development) that it is only in the middle of or late in the first year of life, that appetite as a regulator of food intake appears. By that time, the cerebral cortex has developed enough to permit some of the functions of the mind to become apparent: recognition by sight, smell and tastes of specific foods and feeding situations; perceptual appreciation of the feeding person; presumed memory of the pleasure of past feedings and the presumed anticipation of relief from the discomfort of hunger. Even this level of development of appetite (preverbal) can only be surmised by signs of pleasure such as cessation of crying, cooing or smiling at the sight (smell, touch or hearing) of the mother, breast or bottle. Benedek suggested that this occurs at the fourth to fifth month of life.[57] It is in this general period of the first year of human life that the psychoanalytic and child development literature agrees that ego functions (perception, memory, recognition, anticipation, discrimination, purposeful motor acts, smiling responses, etc.) are just becoming differentiated.

This, at a time when mental functions are appearing, is when appetite as a psychologic drive to eat could be said to begin. I assume that the frequent cycles of hunger–feeding—satiety during the first months of life were somehow perceived, but I cannot regard this as learning or as the development of appetite. I prefer to regard these earliest nutritional life experiences within the physiologic framework of "conditioning" or the ethologic framework of "imprinting." I can only assume with Thorpe[30] that imprinting does register differently if an infant is fed on a strict four hour regime or on self-demand, is cradled in the arms of a loving mother during feeding, or is fed through a propped-up bottle. Such early nutritional experiences, need further study by those who work with babies.

Precision requires that we now assign the term "drive" to appetite, as we have "instinct" to hunger. To me, appetite and appetitive behavior imply purposiveness, aim and goal. The goal is the consummatory act of eating teleologically, to provide essential metabolic requirements of the hunger *instinct*. But

purposiveness, method and aim imply mind and experience. The motives in appetite include a purposeful *drive* to eat for pleasure, as well as to relieve the discomfort of hunger. In making this distinction, I follow Freud's later views as expressed in his paper written in 1915 on "The Unconscious."[58] He wrote, "An instinct can never become an object of consciousness—only the idea that represents the instinct can."[58*] Engel stated this more simply when he wrote, "The mental representation of instinct is called drive."[50] The value of making a clear distinction between instinct and drive is being emphasized in psychoanalytic writings.[59,60,61] I agree with Pleune[62] that instincts have no "aim" whereas "Drives are of a different conceptual level of reality arising only with the development of man's unique psychic apparatus." Such a distinction between instinct and drive in psychoanalysis follows closely that of the ethologists, Tinbergen[29] and Thorpe.[30]

Thus, appetite partakes of drive aims, goals and method, all functions of mind, as distinguished from the hunger instinct. In the first half year of life, before there is adequate cortical development to permit such discrete mental functions, I assume that the anlage of appetite is blurred into all the other undifferentiated faculties of the undeveloped newborn. I can only speculate that whatever perceptions of hunger are registered, are only indiscriminately "felt" as unpleasure and that the gratification of eating and satiety is blended into an opposite "feeling" of pleasure and contentment. In this undifferentiated phase, the discomfort of hunger and the pleasure of adequate feeding are as much *affective states* as nutritional processes. I believe that throughout life, this intimate association of strong feelings of displeasure with hunger and pleasure with appetite and eating are a basic part of nutritional regulations. It is less apparent in the healthy, well-fed adult than it is in the newborn, the starving, the "hungry" wild animal, the limbic stimulated monkey or the cortically injured man.

In a depressed human adult, changes in appetite, accompanying the affective changes, are glaringly apparent. The sequence of emotional upset → hyperphagia in some obese patients is easily demonstrable. Such intimate association between affect and drive (satisfaction or frustration) is receiving increased attention by psychoanalytic writers.[63,64,65] I am inclined to regard the affective components of appetite as one of the "drive affects" (as distinguished from "ego affects") as classified by Engel.[65]

The interpersonal aspects of early nutritional experience must also be emphasized. In the undifferentiated phase of the first half year of life, the infant is literally and completely dependent on another person, usually its mother, for food supplies, and hence for survival. You might say the mother plays a nutritional role as "ego surrogate" for the helpless undeveloped baby. The model for this is the infant suckling at its mother's breast. The mother is as gratified in her role as nutritional provider as the "hungry" infant is in his satiety from feeding. In this repetitive cycle of a mutually gratifying "oral symbiosis," we see the intimate association established between eating, emotional gratification and a developing mother-child relationship. All three components contribute to the development of the child's appetite. Such a mutual feeding experience is as critical for the development of loving object relations and for pleasurable affective living as it is for proper nutrition. For further amplification, see Benedek.[57,66,67] We do not, of course, ignore the many other bodily contacts and "transactions" between infant and mother, but feedings around the clock at two to four hour cycles establishes by experience a rhythm of appetite and an imprinted experience of satiety or frustration, feelings of pleasure or discomfort, which contribute to eating patterns of the adult, as well as many self- and object-directed attitudes and expectations. I submit that this intimate association between appetite, satiety (emotional as well as nutritional) and the relation of an individual to his mother (or her

*For a lucid explanation of the historical confusion which resulted from the earlier translation of Freud's term "Trieb" into "instinct," see Strachey's note introducing Freud's paper "Instincts and their Vicissitudes."[58]

feeding substitute), persists throughout life.

Throughout early childhood, there continues this reciprocal interaction between mother and child in a two-person nutritional activity. The emotional needs and nutritional views of the mother contribute much to the development of her child's appetite. Some mothers will let a child "cry it out" when he may be hungry, until she feels "it is time for him to eat." Other mothers may attempt to feed their children when the child may not be hungry. The child, by virtue of being a child, may feel obliged to comply, unwillingly, to its mother's wishes thus adding a conflicting power struggle between child and parent to the nutritional scene. The child may feel caught between his own instinctive hunger regulations and taste preferences and wanting to please or defy his mother. Out of such ingredients do emotionally determined hyperphagia or hypophagia often arise. Such a possibility is more likely in those households where parents use food and eating as bribes or rewards for good behavior and withholding of favorite foods or even meals as a disciplinary action. In later childhood, the emotional associations to appetite broadens around the family dining table. For a variety of medical, personal, cultural and religious reasons, some families pay special attention to meal time, food and eating. The parents may be diabetic, obese, food faddists, gourmets or follow special religious rituals in the cooking, serving and eating of food. These family attitudes and traditions contribute to the child's appetite and eating patterns so that in later life it may be difficult or impossible for the physician or the nutritionist to modify an adult's eating patterns even for sound nutritional reasons.

In the school period, the formally learned aspects of good nutrition and proper diet further modify a youngster's appetite in courses in personal hygiene and biology. Of course, the appetitive urges at the corner drug store, after school, in the midst of the child's peer group may lead to quite a different food intake!

At puberty, new hormonal adjustments alter the entire energy equilibrium, and body weight may fluctuate markedly. In addition, an emotionally determined, regressive hyperphagia often develops. Oral gratification, hyperphagia and reactive weight gain is often a substitutive method for dealing with the sexual conflicts of puberty.

In adult life, social, medical, cultural, religious and economic forces modify our appetites and largely determine our selection of foods if not the periodicity of our eating habits. Sunday dinner with the family, the religious fast, the coffee break, the business lunch, the cocktail party, the television snack are common examples of such factors in the regulation of food intake. We are now informed that not only the usual advertising pressures of magazines, billboards and television urge us to eat certain foods at specified times and places, but also that subliminal and associative technics of experimental psychology also modify our appetites without our awareness.[22]

It seems likely that, as Carlson wrote in 1912, "Under these circumstances, appetite and habit supplant hunger as nature's dietary guide."[31]

THE CONCEPTUAL YIELD

From the foregoing, I conceive appetite as being a subjective mental process, man's most specialized, complex, regulator of food intake. Appetite integrates the internal metabolic demands of hunger with nutritional realities of the environment: resources, limitations and temptations. As part of the mind, appetite permits voluntary, purposeful and planned eating or not eating behaviors.

Appetite, as a mental process, is a function of the brain. It is, therefore, determined by neuroanatomic associational experiences. The quality and quantity of appetite is multidetermined and can only be fully understood by subjective reports from the subject in addition to objective observations and measurements. As appetite is a conditioned and learned experience, it varies with growth and experience, and each individual appetite has its own ontogenetic history. In its development, appetite is intimately intertwined with all other mental processes including affects, perception, memory, cognition, sym-

bolization, other drive satisfactions or frustrations as well as self- and object relationships. Although we are focused on appetite as a desire for food, we cannot fully understand this nutritional matter apart from other appetites and the success or failure of other drives for gratification. In its broadest sense, appetite is a study of the history of the person's capacities for, experiences with and attitudes toward pleasure and unpleasure.

Appetite in man appears to be unique to the species and to the individual person. Phylogenetically, this specificity relates particularly to the evolution of the cerebral cortical system and the complex interrelations of the limbic and neocortical brain, the site of appetite. Appetite as a mental process can appear only in a mature, intact cerebro-cortical system. Both neurophysiologically and psychologically, appetite represents brain-mind functions at the most mature, specialized integrating level of the multiple regulations of food intake. I assume, as does Jackson,[34] the concomitance of physiologic and psychologic processes at these cerebral cortical levels. In another aspect, I suggest that appetite in man is a nutritional example of brain-mind coordination at the highest integrative levels of the human nervous system. Such specialized, nervous and psychologic control is not available to the newborn human being or to the adult with brain damage. From clinical observations it also appears to me that the nutritional aspects of appetite may be distorted by associated psychologic factors of emotional conflict, immaturity or unsatisfied (non-nutritional) drives or interpersonal gratifications.

Nutritional health could be defined as a relative constancy and integration of appetite with hunger regulations, intake of food and body weight (composition).

Nutritional disease could be defined as a dissociation in the interrelationship between hunger, appetite, food intake and body weight. Such distortions and resulting imbalance can result from manifold disturbances varying from genic, metabolic or hypothalamic, to the quality of the hunger instinct, the functioning of the cerebral cortex, the presence of emotional conflicts, famine or feelings of guilt. These, in turn, may be reflected in a change in oral urges, in subjective desire for food (anorexia, hyperrexia), in food intake (hyperphagia, hypophagia), in physical, mental or emotional expenditure of energy, or in changes in body weight or composition. We must study these complex factors separately.

Two brief examples of such dissociation will, I hope, make the point clear. From my psychoanalytic studies of dreams of food and eating, I already mentioned that one patient (1) had a higher incidence of these dreams (which I regard as a reflection of "oral urges") than did another patient (2) (Table 1). Neither woman had disturbances in food intake or body weight. This finding, in context with other data from the two psychoanalyses, makes me believe that the first patient (1) has a greater psychologic predisposition to hypophagia or hyperphagia and secondary inanition or reactive obesity than did the other patient (2). Such a finding (though not necessarily valid for this particular patient nor this particular comparative interpretation) illustrates the need to study oral urges apart from altered eating behaviors or weight changes. When refined, I hope such studies can be used like the glucose tolerance test or the plasma pepsinogen test to define certain predispositions to particular nutritional diseases. This would be in keeping with Mirsky's formulations.[28]

A second illustration of nutritional dissociation is taken from Stunkard's report concerning the similarity of gastric peristaltic activity patterns (hunger) in the empty stomachs of obese and non-obese women.[6] The subjective reports as to "hunger, emptiness and desire to eat" (appetite) differed markedly in the two groups. In my discussion,[69] I suggested that there was a dissociation between the gastric hunger and appetite regulations. Both Stunkard and I agreed that the pathology lay in "faulty perceptions" of the group of women who were obese although he referred to it as "denial of hunger," and I as the "psychopathology" of their appetites.

In this paper I have concentrated attention on the clinical psychopathology of appetite and the neuropathology of the cerebral cortex.

In the common nutritional disorders of man, I have the impression that these areas are often affected. I believe this relates to the specialization and complexity of these "higher center" regulations of food intake. I postulate that they are definitely susceptible to noxious insults of many types, more so than the hunger regulations at the gastric or hypothalamic level. I believe we need to keep in mind Jackson's remarks on the "dissolutions" of the nervous system: "When we consider nervous maladies as Dissolutions we have to bear in mind not only the Dissolution, that which is effected by disease in the sense of pathological change, but also the Evolution going on in the undamaged healthy, remainder."[24] This principle appears to be substantiated in the case reports of adults with damage to the cortex whose stomachs, for example, have been found to have excessive gastric peristaltic activity and symtomatically express "morbid hunger," bulimia or weight gain.

The psychopathology of appetite, for example psychologic conflict, depressive feelings or ungratified longings for a loved person, is often the precipitating event in nutritional disease. Such psychopathology is a frequent and major factor in the disruption of healthy regulations of food intake.

SUMMARY

The concepts of hunger and appetite, as regulators of food intake in man, are compared and contrasted. It is proposed that the term hunger be applied only to biochemical and physiologic processes; appetite only to psychological regulations. Hunger is discussed as an instinct, appetite as a drive, and both at varying levels of consciousness. Appetite subsumes all affective, perceptual, ideational and conative regulations of food intake. Appetite, as a function of man's mind, is interposed between the internal, metabolic demands of hunger and appetitive and consummatory behaviors directed to the environment.

The regulation of food intake in man can be fully understood only if man (as adult and child) is studied. This is due to man's phylogenetically unique cerebral cortex which permits mental representations of drives and, therefore, introspective, subjective perceptions of appetite which must be included in the study of nutritional regulations. Emphasis is given to limbic and neocortical aspects of the regulation of food intake by concomittant and integrated physiologic and psychologic processes.

Because man's mind allows delays, distortions and substitutions of drives, the manifest nutritional behaviors of hyperphagia and hypophagia are often a substitute gratification for other needs and drives which are nonnutritional in origin. Such distortions and substitutions are termed the psychopathology of appetite. These mechanisms usually occur at unconscious levels of awareness.

Because man's mind permits symbolization of foods, eating behaviors and certain viscerosomatic processes, the author's studies of dreams of food and eating were elaborated. These appear to be a useful method of psychoanalytic research for studying unconscious aspects of oral urges, food and eating behaviors.

Emphasis is given to studying separately metabolic needs, oral urges, food intake and eating behaviors in order to delineate separate components in the regulation of food intake. The need for continuing multidiscipline investigations, with new and more refined methodologic approaches, is apparent.

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Biochemical Concomitants of Hunger and Satiety in Man

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ALTHOUGH gastric distention is known to influence the quantity of food eaten, a variety of studies have demonstrated that the stomach is dispensable in so far as the control of food intake is concerned.[1] Within certain limits, animals will increase the volume of food ingested to compensate for dilution of the diet with calorically inert substances.[2,3] Animals with denervated gastrointestinal tracts show normal regulation of food intake.[4]

For these and other reasons, attention has been directed toward the metabolic events that accompany and possibly influence states of hunger and satiety in man.[5,6] A common sense theory has arisen that during hunger, depletion of some component of the blood stimulates the "hunger center," and after a meal, repletion of this same component inhibits hunger. Actually, there is no *a priori* reason to believe that a constituent of the blood must be depleted during hunger; instead such a constituent might increase and exert an effect in this way. Because the concept of one satiety signal appeals to our sense of parsimony, we should not overlook the possibility that several such signals may be operative.

One approach to the problem of discovering what the biochemical changes in blood affecting the food regulatory center are, would be to record, under natural conditions of food consumption, the status of subjects as to hunger or satiety and at the same time to measure a variety of biochemical parameters in the blood.

When a meal containing normal proportions of fat, carbohydrate and protein is consumed, certain characteristic changes occur in the blood and the metabolic state. Some of the changes are shown in Table I. These lists are not complete, but are presented to distinguish between the early consequences of food ingestion; namely, an increase in the level of certain constituents in blood, and the changes in metabolic state that occur as a result of these primary events. Any change, primary or secondary, could act as a signal to the food-regulatory center; nevertheless, secondary events have to be thought of in continuity with the primary phenomena upon which they depend.

The various metabolic events that occur after a normal meal is consumed have led to formulation of a number of theories concerning the regulation of food intake[6] (Table II). Of these, the glucostatic theory[7] is of primary interest in relation to the studies reported herein.

This theory proposes that the available supply of carbohydrate in the body exerts a regulatory influence on the intake of food via hypothalamic "glucoreceptors," the urge to eat increasing as stores of carbohydrate diminish. The hypothalamus probably can not obtain direct information concerning the status of the carbohydrate stores; however, the rate at which carbohydrate is utilized by the body as a whole tends to be directly

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TABLE I

Metabolic Events Occurring in Association with Ingestion of a Conventional Meal

- Primary changes (in blood constituents)
  - Increased
    - Glucose
    - Amino acids
    - Lipids
      - (Low density lipoproteins-chylomicrons)
- Secondary Changes
  - Increased
    - Glycogen-liver, muscle
    - Lipogenesis
    - Chylomicron deposition in adipose tissue
    - Specific dynamic action
    - Respiratory quotient (rises)
    - Insulin secretion
    - TPNH, etc.
  - Decreased
    - NEFA—release rate
    - Lipolysis
    - Ketogenesis
    - Gluconeogenesis
    - Secretion of:
      - Epinephrine
      - Growth hormone
      - Glucocorticoid

proportional to the supply of this nutrient. Thus, measurement of the rate at which blood glucose is utilized (peripheral arteriovenous glucose differences) provides indirect information about the available supply of carbohydrate.

In past attempts to test the glucostatic theory, studies were performed to determine whether a temporal association could be disclosed: (1) between the experience of hunger and a diminished rate of peripheral glucose utilization and; (2) between satiety and an increased rate of glucose utilization.[11–13] Several difficulties are inherent in the correlation of differences of arteriovenous glucose with hunger satiety states. First, measurement of peripheral differences of any metabolite, if the results are to be interpreted in even a partially quantitative fashion, must be accompanied by accurate determination of the flow of blood through the part under study. Second, local changes in the uptake of glucose, such as may occur in the distal portion of an extremity, may not be representative of the general metabolic behavior of extrahepatic tissues. Third, peripheral arteriovenous glucose differences cannot be measured in small laboratory animals.

TABLE II

Theories Concerning the Regulation of Food Intake

Modality	Theory	Signal*
Chemical	Glucostatic[7]	Availability of blood glucose
	Lipostatic[8]	Concentration of circulating metabolite (nature unspecified)
	"Amino acid pattern"[9]	Pattern of available amino acids in blood
Physical	"Thermostatic"[10]	Specific dynamic action of ingested food
Neural	"Liponeurostatic"[5]	Impulses from fibers innervating adipose cells

* To a regulatory center in the hypothalamus.

Since the organism increasingly must derive energy from fat when the carbohydrate supply diminishes, the level of non-esterified fatty acids (NEFA) in the blood might provide indirect information concerning the rate at which the body is utilizing glucose. Several groups of investigators[14,15] have shown that NEFA levels in the plasma tend to fall when blood sugar rises, and vice versa. Such behavior might be expected if the adipose tissues release more NEFA as the glucose supply diminishes. However, for plasma NEFA to rise, the rate of release of these fatty acids would have to exceed the rate at which they are used.

In view of these considerations, studies were undertaken to determine the following: (1) whether a significant correlation could be discerned between variations in peripheral arteriovenous glucose differences (as they occur diurnally in normal subjects on self-selected diets) and changes in plasma NEFA levels; (2) whether an association exists between the pattern of plasma NEFA in time and concurrent experience of hunger or satiety and; (3) whether such associations or correlations could still be obtained when meals of different composition were consumed.

MATERIAL AND METHODS

Three experiments were performed. (1) Nine subjects consumed their usual, self-

TABLE III
Means and Standard Deviations of Caloric Values and Constituents of Meals During Experiments

Meal	Calories	Carbohydrate (gm.)	Protein (gm.)	Fat (gm.)
Regular Diet				
Breakfast	643 ± 141	75 ± 25	23 ± 7	30 ± 8
Luncheon	1,003 ± 160	105 ± 47	46 ± 14	56 ± 12
Carbohydrate-Poor Diet				
Breakfast	838 ± 42	...	49 ± 22	71 ±26
Luncheon	1,055 ± 151	...	73 ± 28	84 ± 36

selected diet and were asked to record what was eaten and the time and duration of breakfast and luncheon on the experimental day. (2) Six of the same subjects and one additional subject were then changed to a regimen that was virtually free of carbohydrate and in which breakfast and luncheon consisted of meat, butter, eggs and fish, in self-selected amounts. (3) In the third experiment, a man,* aged seventy-nine, who had voluntarily consumed a carbohydrate-poor diet for five years, was studied during a typical day over a period of nine hours that included a self-selected carbohydrate-poor breakfast and luncheon.

In all three experiments, plasma NEFA levels and capillary (arterial)[16] and antecubital venous glucose concentrations (C-V glucose differences) were measured while the subjects were in the postabsorptive state, at thirty, sixty and ninety minutes after breakfast, before luncheon, and at thirty, sixty and ninety to one hundred eighty minutes after luncheon.

The subjects were permitted to eat luncheon only after some degree of hunger had returned.

The data on the self-selected meals are given in Table III.

Degree of hunger present immediately before each sample of blood was drawn was estimated by asking the subject to read the following questionnaire containing descriptions of four gradations† of hunger intensity,[17] and to select the statement that best described his own condition: (1) no desire to eat; (2) could eat but don't want to; (3) moderate desire to eat and; (4) strong desire to eat.

Plasma NEFA concentrations were measured by the method of Dole,[15] and blood glucose levels were determined by the Nelson-Somogyi procedure.[18] Venous blood was obtained from an antecubital vein without stasis, and capillary blood was pipetted from a finger tip following cutaneous puncture. All determinations were performed in duplicate.

RESULTS

The subjects, who consumed self-selected conventional meals, showed rises in capillary and venous glucose levels after breakfast. These levels usually returned to or near baseline levels within ninety minutes (Fig. 1). The capillary glucose concentration always increased to a greater degree than did the venous glucose concentration; hence, appreciable C-V glucose differences tended to occur at thirty, sixty and ninety minutes after the morning meal. At the same time C-V glucose differences were increasing, NEFA levels were falling in reciprocal fashion.

Sixty to 120 minutes after breakfast, C-V glucose differences began to decrease and returned to postabsorptive levels until after the mid-day meal. As C-V glucose differences decreased, NEFA levels rose, sometimes exceeding the fasting levels of the morning.

All of the subjects described some degree of hunger before breakfast. Immediately after breakfast, hunger diminished or was abolished for approximately ninety minutes, but gradually returned and was usually clearly manifested, three to four hours after breakfast.

After luncheon, changes in C-V glucose differences, NEFA and hunger intensity were similar to those observed after breakfast.

* The authors acknowledge the cooperation of Vilhjalmur Stefansson, Arctic Consultant, Dartmouth College, who served as a volunteer subject in this experiment.

† Since hunger and satiety are subjective states, the difficulties inherent in defining and grading them are obvious.

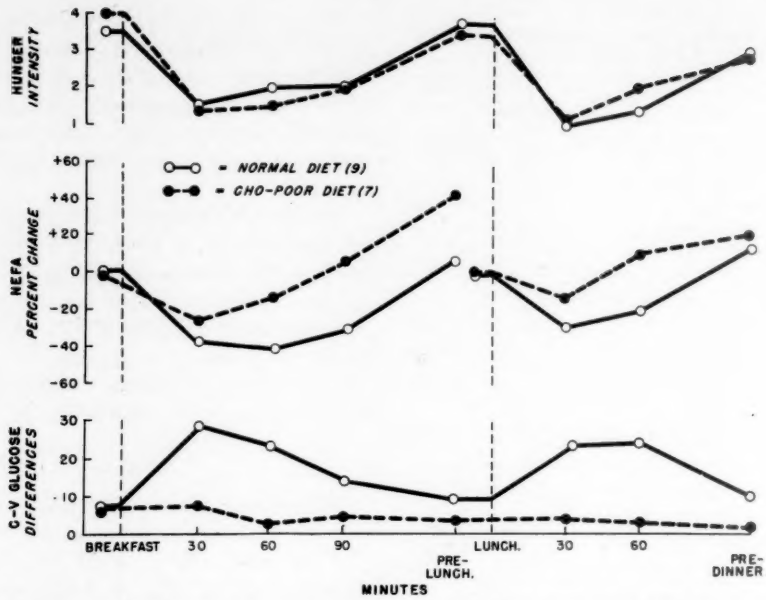

FIG. 1. Mean changes in hunger intensity, non-esterified fatty acids (NEFA) and capillary-venous (C-V) glucose differences in healthy subjects in response to (1) a "normal" breakfast and luncheon, and (2) to a carbohydrate-poor breakfast and luncheon. (NEFA after luncheon are calculated as per cent change from preluncheon value.)

The seven subjects, who consumed a carbohydrate-poor breakfast and luncheon, showed no change in either capillary or antecubital venous glucose levels after ingestion of meals. As a consequence, peripheral C-V glucose differences remained small or negligible throughout the experiment (Fig. 1).

NEFA levels fell slightly after breakfast and luncheon in this group of patients, but the duration of the fall was shorter and the rebound greater than was noted in the group who ate regular meals. Several subjects showed no drop in NEFA levels after consuming a carbohydrate-poor luncheon.

All of the subjects on the carbohydrate-poor diets were hungry before breakfast and experienced satiety after ingesting the carbohydrate-poor meal. Hunger was abolished or diminished according to a pattern similar to that observed for the subjects on a regular diet. The same pattern was obtained after luncheon except that the duration of satiety in the carbohydrate-poor group was shorter.

The results for one subject, who consumed a carbohydrate-low breakfast and luncheon, are presented separately (Fig. 2). This person had been on a carbohydrate-poor diet for approximately five years and was accustomed to this type of meal.

The subject consumed a breakfast consisting of two eggs and eight pats of butter. The meal was followed by loss of hunger for approximately four hours and the subject expressed willingness to eat four and one-half hours after breakfast.

Blood glucose values for this subject remained fairly constant with capillary levels rising slightly after breakfast. C-V glucose differences remained negligible. NEFA levels fell slightly during the first hour after breakfast and then rose rapidly to high levels that were maintained through the luncheon period. One hour after completion of luncheon, the NEFA concentration was at its highest, approximately 3 m.Eq. per L., and remained near this point throughout the afternoon.

Luncheon consisted of one-half pound of steak including the fat edge, two pats of butter

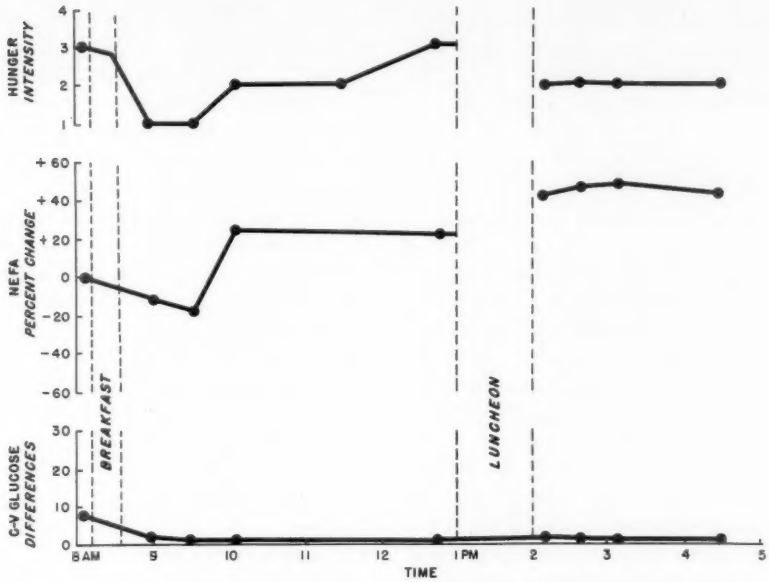

FIG. 2. Changes in hunger intensity, non-esterified fatty acids (NEFA) and capillary-venous (C-V) glucose differences in one subject in response to a carbohydrate-poor breakfast and luncheon. (NEFA after luncheon are calculated as per cent change from preluncheon value.) The subject (V. S., aged seventy-nine) had voluntarily consumed a carbohydrate-poor diet for five years.

and two ounces of sour cream. This meal was followed by a decrease in hunger intensity which persisted until the last samples of blood were drawn three and one-hall hours later.

COMMENTS

In these experiments an attempt was made to determine the following: (1) the extent to which the diurnal hunger satiety pattern might be affected by an abrupt change in the composition of a meal; (2) whether NEFA changes might show a satisfactory inverse correlation with peripheral arteriovenous glucose differences and; (3) whether a person accustomed to a carbohydrate-poor diet would show different diurnal hunger satiety patterns and different patterns of NEFA and blood glucose change than would be observed in the group shifted abruptly to a similar carbohydrate-poor diet.

The results show that an abrupt and drastic change in the composition of a meal affects the diurnal hunger satiety pattern only to a slight degree. For example, in the group on the carbohydrate-poor diet, the satiety experienced after the mid-day meal was of shorter duration. Whether this difference would have statistical significance in a larger series cannot be stated at this time.

Previous studies on the physiology of NEFA have suggested that plasma NEFA levels vary inversely to the rate of utilization of glucose.[14,15] Since peripheral C-V glucose differences also provide an index of rate of extrahepatic utilization of glucose, it was of interest to determine how these two measurements correlated with each other.

When the experimental subjects were consuming self-selected meals, containing appreciable amounts of carbohydrate, there was a good inverse correlation between NEFA levels and C-V glucose differences. On the other hand, when the same subjects consumed carbohydrate-poor diets, NEFA levels continued to fall to some extent after meals, even though fluctuations in blood sugar were minimal. This observation is consistent with

a recent report that the level of amino acids in the blood may have an influence upon the rate of NEFA release.[19]

The subject, accustomed to a carbohydrate-poor regimen, showed a similar metabolic response to that of the subjects changed abruptly to the same regimen. This person showed the same flat glucose curves seen in the unacclimated group. The high NEFA rise during the day may have reflected an increased ability to mobilize fatty acids.

It may be asked whether the metabolic events observed play any etiologic role in hunger and satiety, or whether they are merely associated phenomena. The data do not provide an answer to this question.

The peripheral C-V glucose differences did not correlate well with hunger satiety patterns in subjects on carbohydrate-poor diets. This observation supports the view that satiety is a complex phenomenon with a number of components. There is some preliminary evidence that when carbohydrate is absent from a meal, the duration of subsequent satiety may be curtailed.

NEFA changes tend to parallel the hunger-intensity curve regardless of the composition of the diet. In this regard, the rate of release of NEFA may be responsive to the intake of dietary protein as well as to the intake of carbohydrate.

SUMMARY

Studies were performed to determine the following: (1) whether a significant correlation could be obtained between diurnal changes in peripheral arteriovenous glucose differences and variation in plasma non-esterified fatty acids (NEFA) in normal subjects on self-selected diets; (2) whether an association might exist between the diurnal changes in plasma NEFA and concurrent experience of hunger or satiety and; (3) whether a similar association could be obtained when meals of altered composition were consumed.

Nine subjects consumed their usual self-selected diet and a record was made of what was eaten during breakfast and luncheon. Six of these subjects and one additional subject were changed to a breakfast and luncheon that were free of carbohydrate. One subject, aged seventy-nine, who had voluntarily consumed a carbohydrate-poor diet for five years, was studied during a typical nine hour day. Measurements were made of plasma NEFA and capillary (arterial) and antecubital venous glucose concentrations while the subjects were in the postabsorptive state, and at half to one hour intervals following their meals.

In the subjects on self-selected mixed diets, a good correlation was obtained between diurnal changes in arteriovenous glucose differences and NEFA. Appreciable increases in arteriovenous glucose differences were associated with reciprocal changes in NEFA levels. The hunger satiety pattern paralleled the pattern of NEFA change.

When the subjects were changed to carbohydrate-poor diets, arteriovenous glucose differences remained negligible throughout the day. The change of pattern of NEFA, however, tended to parallel the hunger satiety pattern. The subject, who was accustomed to a carbohydrate-poor diet, showed a similar response.

It is probable that NEFA levels *per se* do not act directly as a signal to the food regulatory center. However, the pattern of NEFA change may provide a useful index of the physiologic readiness of the body to consume more food.

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DISCUSSION

DR. ESTELLE R. RAMEY, (*Washington, D. C.*): It is true that under most circumstances a drop in the blood glucose will be reflected in changes in the non-esterified fatty acid (NEFA) levels (if you administer a good deal of glucose, you can obtain a drop in the NEFA levels) except under the circumstances of the administration or production of the catecholamines. Epinephrine will produce a hyperglycemia and also a high NEFA level.

The neurogenic effects on NEFA levels may be quite independent of the caloric state of the animal at a given time.

We have found that it is difficult to use NEFA levels as an index in animals, for example, who are excited, so to speak, shortly before the sample of blood is removed.

DR. VAN ITALLIE: The effect of stress is to confuse the NEFA picture.

In general, when carbohydrate is administered, however, it does, as has been shown by Duner and others, suppress the secretion of epinephrine, provided there are no other neurogenic factors operative.

DR. JEAN MAYER, (*Boston, Massachusetts*): We find that the effects on food intake of either epinephrine or norepinephrine are similar and they continue in the same manner after the ventromedial area has been removed.

I wonder whether in regard to hunger behavior, the effect of epinephrine or norepinephrine is purely neurologic and not mediated through metabolic changes, no matter how profound these may be.

DR. MAURICE RABEN, (*Boston, Massachusetts*): In regard to this value of 3,000 mEq. per L. of fatty acids of Mr. Stefansson, is there anything strange about Mr. Stefansson except that he eats steak and eggs for breakfast?

Have you, or Dr. Dole, or anybody else seen values of this sort in subjects without diabetes except for Mr. Stefansson.

DR. VAN ITALLIE: We have never seen values this high in subjects without diabetes.

Mr. Stefansson is an individual with a strong countersuggestibility. The time that the President of the United States had a coronary thrombosis and everyone was bemoaning the fact that the American public was eating too much fat, Mr. Stefansson recalled the fact that he had lived comfortably on seal on the Beaufort Sea and on pemmican, and decided he was going to go on a carbohydrate-free diet except for an occasional old fashioned, or martini. He decided that alcohol is not a carbohydrate.

In terms of his state of health and vigor, there is nothing strange about him except that he is so remarkably agile and energetic. As far as his serum cholesterol level is concerned, for example, it was 280 mg per cent when we measured it. And his blood was not grossly lipemic, although there was a modest amount of lipemia after meals. The fasting samples were entirely clear.

DR. VINCENT P. DOLE (*New York, New York*): We have measured an enormous number of non-esterified fatty acids and I have never seen one so high as this except in a person with diabetic ketosis or at the peak of a response to a dose of epinephrine. But in any of the average population one might see, this is unprecedented.

As a matter of fact, it is so large that it raises the question as to how the non-essential fatty acids were carried, because it will work out perhaps to about three molecules of fatty acid per albumin and we know that under normal conditions, at least, the non-esterified fatty acids are virtually or entirely bound to albumin. It is possible some of these spill over onto the lipoproteins, as some of the NIH group have suggested with excess amounts of non-esterified fatty acids.

It would be of interest to study the electrophoretic

pattern of his lipoproteins and see whether, in fact he has any abnormalities of the sort that Gordon observed on adding fatty acids to the plasma.

The second facet is whether this patient has ketosis. We have entertained the theory that the source of ketosis in the patient with diabetes is an excess release of fatty acids and overloading the liver with substrate. If high level of non-esterified fatty acids in this patient is due to an abnormally high rate of release rather than some adaptive change in the mechanism for taking up non-esterified fatty acids in the tissue, then it would seem that he might be subject to ketosis. What his state is as to the ketone bodies in the urine.

DR. VAN ITALLIE: We did not measure the ketone bodies. We used method of Dole for measuring non-esterified fatty acids. I feel it is reasonably accurate because our other values were so much lower. This was an unusual finding for us.

DR. BARBARA MOULTON (*Washington, D. C.*): The Secretary of the Department of Health, Education and Welfare, in a press release two or three weeks ago, made the statement that there was not any single simple, safe food, drug or device which knocked appetite out without any exercise of will power, and Mr. Stefansson took public issue in a letter to The New York Times, claiming that his diet, this high meat fat diet with no carbohydrates, did just that, knocked appetite without any will power.

I am interested in the comment by Dr. Van Itallie that the duration of satiety was lessened in Mr. Stefansson more than in any of the other subjects. Has that particular observation been repeated?

DR. VAN ITALLIE: I think Mr. Stefansson is an unusual man in a variety of ways. As far as his will power is concerned, it seems to be enormous. But I cannot answer your question further.

We chose him because he was the only person we knew who was accustomed to this kind of diet.

A Method of Studying Physical Activity in Man

ALBERT STUNKARD, M.D.*

THIS PAPER describes a method of studying physical activity in man, and some of the limitations and potentialities of this method. The instrument utilized for measuring activity is the mechanical pedometer.

Despite the attested value of the measurement of physical activity in laboratory animals, and despite the paucity of such measurements in clinical studies, there are surprisingly few reports of investigations which utilized the pedometer. In 1926 Lauter[1] described his own physical activity as measured by a pedometer, but reported little more than his surprise at the relatively large degree of activity recorded in the course of a sedentary occupation. In 1949 Larsen[2] reported that a group of hospitalized obese persons walked less than a group of non-obese persons as measured by the pedometer. Finally, two investigators attempted to describe patterns of physical activity corresponding to phases of the menstrual cycle with, unfortunately, contradictory results.[3,4]

For the past four years we have been using the mechanical pedometer for studies of physical activity in man. This instrument, the size of a pocket watch, is inexpensive, easy to use and relatively reliable. It functions on a simple principle. A delicately balanced arm is displaced by slight jolts in the vertical plane and these displacements turn a series of gears. When the pedometer is worn suspended from the waist each step taken during walking transmits an impulse to the balance arm. By calibrating the pedometer for the length of stride of its user it is possible to convert such impulses into distances. Standardization of the pedometer by subjects walking a measured mile indicates that it can measure distances walked with an error of less than 15 per cent.

PROBLEMS

There are three types of problems in the use of the pedometer. The first problems are those of the instrument itself, the second are the uncertainties as to what it measures and the third are the difficulties in interpreting these measurements.

Currently available commercial pedometers are relatively fragile and their accuracy is readily impaired by events incident to their use. Dropping a pedometer, for example, may break it outright, while the entrance of dust and moisture through the loosely fitted case sooner or later interferes with the performance of all pedometers. It is, therefore, necessary to retest pedometers at frequent intervals. Since such testing is most satisfactorily carried out by the subject walking a measured mile, considerable cooperation is needed for long-term studies.

Despite its relative accuracy in measuring distances walked over a measured mile the pedometer has limitations both as a direct measure of distances walked and as an indirect measure of caloric expenditure. Bodily movements other than walking may move the balance arm and thus result in an exaggeration of the distances walked. Since these movements are a measure of the total activity of the

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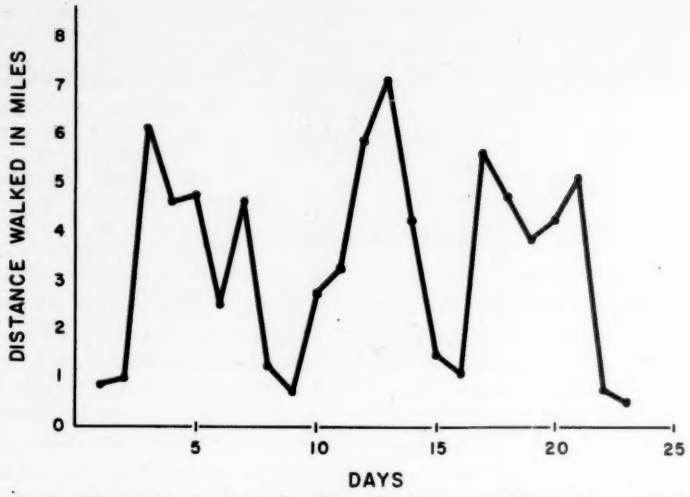

FIG. 1. Fluctuations in physical activity of a thirty-year old man over a period of twenty-three days. On the ordinate are measured distances walked in miles, on the abscissa, time in days. Note the marked variability of the data.

subject, however, recording them may actually increase the precision of estimation of caloric expenditure. It should be borne in mind, nevertheless, that distances walked in a pedometric study serve as an index of physical activity rather than as a precise measure of actual distances walked. Another lesser problem is that bodily movements, such as those of the extremities, which do not affect the pedometer, nevertheless result in at least some degree of caloric expenditure.

The third problem in the use of the pedometer arises from the great variability of the data which it measures. In contrast to the remarkable regularity in the patterns of physical activity of some of the lower animals, physical activity in man follows a course so irregular as to appear at times almost random. Figure 1, for example, is a record of the daily physical activity of a thirty-year old man over a period of twenty-three days. By contrast, Figure 2 shows a plot of twenty-three consecutive numbers from a table of random numbers. After some years of study, we have come to believe that this apparent randomness of the data in man is simply an expression of the many factors which determine his physical activity. In our work we have tried to deal with this problem by using two kinds of studies.

The objectives of the first type of study were to identify the various determinants of physical activity in man and to ascertain the relative contribution of each. If these are achieved it should be possible to factor out the influence of each determinant as it is identified, thereby reducing the apparent randomness of the data and rendering more evident the influence of factors which have not yet been identified. The purpose of the second type of study was to compare groups of persons, keeping constant as many determinants as possible and seeking differences between the groups in the factor under investigation. The first type of study is primarily exploratory and hypothesis-forming, the second one, confirmatory and hypothesis-testing.[5] Examples of each of these types of studies follow.

EXPLORATORY STUDIES

The basic data for exploratory studies were obtained from the daily pedometer readings of patients who were undergoing psychotherapy and of colleagues who were keeping diaries. We simply asked these subjects to record every night the distance they had walked during the day. It was possible by this means to accumulate large amounts of information in a short time, and in a very economical way.

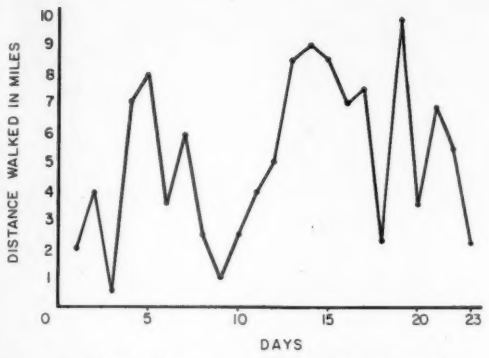

FIG. 2. Fluctuations of a series of random numbers. The similarity of this plot from a table of random numbers to the pattern in Figure 1 emphasizes the apparently random quality of human physical activity.

We had available for analysis, for example, measures of the daily activity of fourteen subjects over periods of from four to thirty-six months.

We began the analysis of these data by trying to set down all the possible determinants of physical activity. Then we examined the data, considering the evidence for each of the possible determinants. Using our current scheme of organization, which is largely an *a priori* one, we divided the determinants of physical activity into three main groups: the "biological," the "social" and the "emotional."[6]

Under "biological" determinants, the following five factors which influence the activity of lower animals have been listed.

(1) *Physical illness* and *injury* play as important a role in the physical activity of man as in that of the lower animals. Indeed, these relationships are so constant as to suggest that a measure of physical activity might profitably supplement standard clinical procedures. The physical activity of two cardiac patients, for example, has proved as sensitive a measure of the status of their congestive failure as have such clinical tools as heart rate and vital capacity.

(2) *Body weight* appears to play a role similar to that in lower animals, in that excessive body weight decreases physical activity.

(3, 4 and 5). Three determinants of physical activity in lower animals *environmental temperature, phase of the female reproductive cycle* and *alterations in food intake*, did not appear to affect our subjects. Man may well be susceptible to such influences but other factors, primarily social and emotional, appear so much more potent as to obscure their effects.

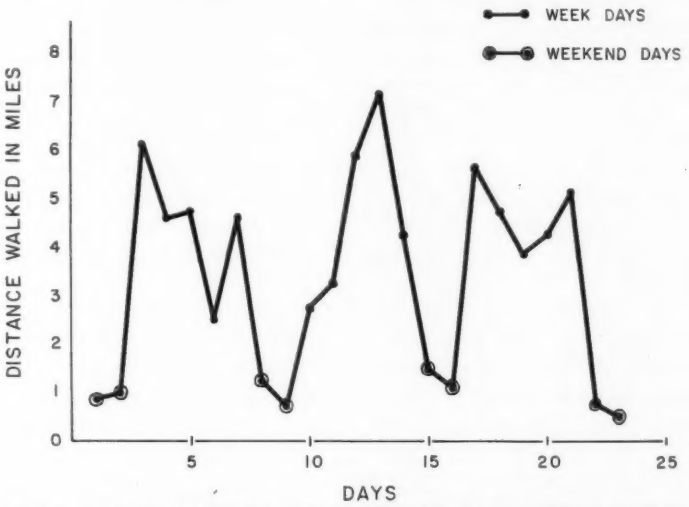

FIG. 3. Variations in physical activity associated with occupational requirements. On the ordinate are measured distances walked in miles, on the abscissa, time in days. The apparently random pattern of Figure 1 is seen to be in part a function of differing amounts of physical activity on weekdays and weekends.

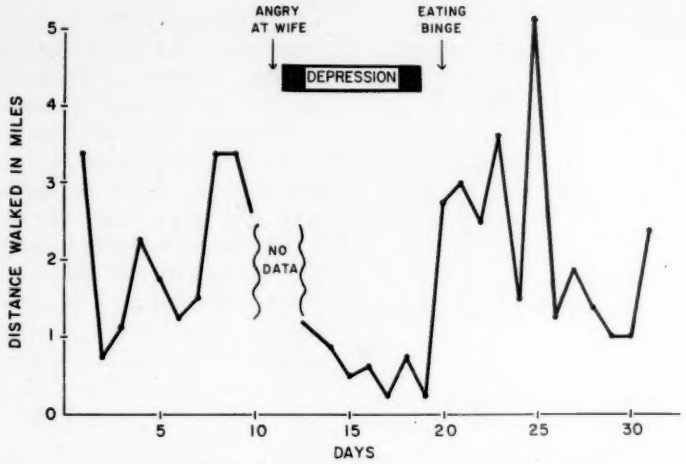

FIG. 4. Decreased physical activity in the course of a depressive reaction. The subject is the thirty year old man, whose activity was shown in Figures 1 and 3, and the scale is the same as in those figures. From an average activity of 2.1 miles per day, the subject fell to 0.5 miles per day, returning to an average of 2.1 miles per day with clearing of the depression. This episode is described elsewhere at greater length.[6]

"Social" determinants of physical activity may be defined as those factors which are derived from the requirements of one's social role. Among physically healthy persons the influence of occupational status can be the single most important determinant of physical activity. This influence may be noted in Figure 3, which presents the apparently random data on physical activity shown in Figure 1. It will be noted, however, that on the weekdays when the subject was busy at work his physical activity was four times greater than that on the weekends when personal preference largely determined his degree of physical activity. Such variations are not found in every person, and people who show them at one time may not at another. But the marked influence of occupation on physical activity emphasizes the need for careful matching for occupation in comparisons of groups of subjects, and for separate analysis of weekday and weekend data on longitudinal studies of individual subjects. Furthermore, the apparent precision of pedometer readings in measuring these differences suggests that the instrument may be of value in classification of occupations.

Under "emotional" determinants, have been classified all types of individual reaction patterns to situations causing stress. A wide variety of environmental stresses seem to decrease physical activity in man, one reaction pattern in obese persons is particularly associated with decreased physical activity. This is depression. Among our obese subjects, almost every episode of depression for which pedometer measurements were available was associated with decreased physical activity. A short-term depressive reaction with decreased physical activity is illustrated in Figure 4.

Most of our observations have been of obese persons; we do not know if this finding of decreased physical activity during depression applies to non-obese persons, as well. Recently, we observed two episodes of depression in non-obese persons which were not characterized by decreased physical activity. This raises an intriguing possibility: is a special kind of depressive reaction, one characterized by decreased physical activity, a pathogenic factor in obesity?

HYPOTHESIS-TESTING STUDIES

Studies carried out in collaboration with Dorris[7] and with Chirico[8] have tested the hypothesis that decreased physical activity

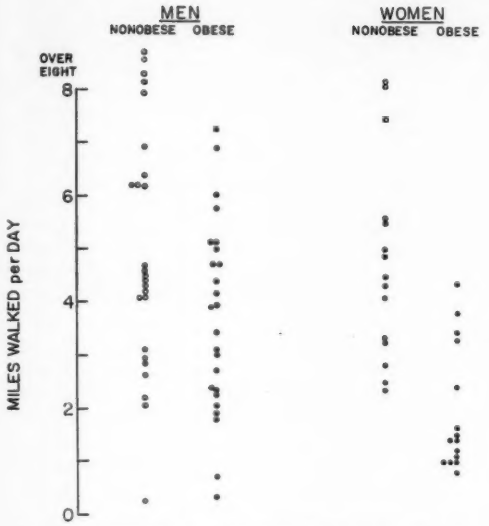

FIG. 5. A comparison of the physical activity of obese and non-obese men and women. Each point represents the average distance walked per day for each subject.

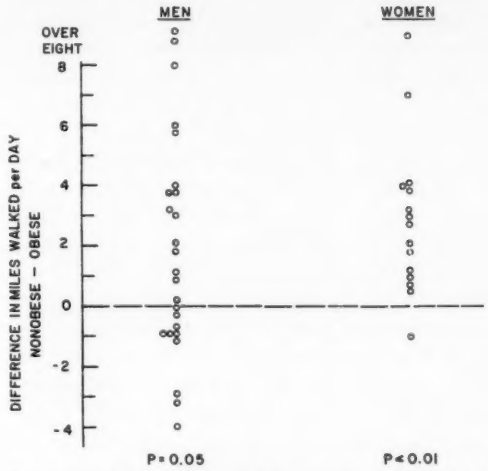

FIG. 6. Further demonstration of the difference in physical activity between obese men and women when compared with non-obese control subjects. (See text.)

plays a role in human obesity.[9–12] Both of these studies were carried out on outpatients from the general medical clinic of a large teaching hospital. Fifteen obese women and twenty-five obese men were matched with respect to age and occupation with an equal number of non-obese women and men from the same clinic. Neither group contained persons with illnesses which might impede activity; the selection was otherwise a random one. Median per cent overweight of the obese women was 52, of the men, 54 per cent. Each subject wore a pedometer for a period of one or two weeks and the distance walked per day was used as the basis of comparison.

The results of the study of women were striking and unequivocal: the obese women were far less active than their non-obese control subjects (Figs. 5 and 6). Figure 5, which shows the distribution of physical activity among the various subjects, reveals that ten obese women walked less than 2 miles per day, a shorter distance than was walked by any of their non-obese control subjects. The mean activity of the obese women was 2 miles per day as compared with 4.9 miles per day for the non-obese women. Since the population on which these observations were made was not normally distributed, and since matched pairs were studied, the data were analyzed by the Wilcoxon matched-pairs signed-ranks test.[13] The data used for this calculation are illustrated in Figure 6. This figure shows the differences in physical activity between each pair of subjects. If there were no differences, the point representing one pair would fall on the 0 line. If the non-obese subject walked farther the point falls above the line, if the obese subject walked farther the point falls below the line. Only once in fifteen times did the obese woman walk farther than her non-obese control subject. The difference between groups is significant at the 1 per cent level of probability.

A study of the physical activity of men revealed differences between obese and non-obese subjects which, although statistically significant, were far less striking than the differences between obese and non-obese women. Figure 5 illustrates the similar distribution of the physical activity of obese and non-obese men and contrasts it with the marked difference in the activity of obese and non-obese women.

The mean activity of the obese men was 3.7 miles per day as compared with 6 miles per day for the non-obese men. Although this difference between the means is nearly as

great as that found with the women, it is deceptively large because of the disproportionate effects of four extraordinarily active non-obese men. A more valid comparison is that illustrated in Figure 6 which shows the differences in physical activity between pairs of subjects. It will be seen that non-obese men usually walk farther than their obese partners, and the Wilcoxon test reveals that this difference is significant at the 5 per cent level. However, the difference between obese and non-obese groups is far less striking among men than among women.

Since obese persons must expend more calories than non-obese persons to perform the same amount of activity,[14] the caloric expenditure of obese men due to physical activity may be fully as great as that of non-obese men. One might conclude from this study that whereas decreased physical activity may frequently play a role in the obesity of women, it does so only rarely in men. The origins of obesity in men, thus, may be sought more profitably in the area of food intake.

SUMMARY

A method for the measurement of physical activity in man is described. Such activity shows great variability as compared with the activity of the lower animals. It also appears to be the result of a wide variety of determinants, ranging from what might be called "biological" to "social" and "emotional" ones. Measurement of the physical activity of obese women reveals that they are far less active than non-obese women. A study of men, on the other hand, demonstrates a significant but far less striking difference in physical activity between obese and non-obese subjects.

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DISCUSSION

DR. JEAN MAYER (*Boston, Massachusetts*): I would like to confirm some of Dr. Stunkard's statements about the difference in obesity between men and women, at least in this particular culture. We find that in adolescent girls, again inactivity is an extraordinarily prevalent condition among obese subjects.

About five or six years ago we carried out a study in Brookline, and we have made confirmatory studies in other communities in Massachusetts. Generally we find that the food intake of adolescent girls who are obese is less, on the average, than the food intake of non-obese adolescent girls. However, the first law of thermodynamics is not flouted, because if you look at the other side of the balance sheet, you find that the degree of inactivity of the obese adolescent girls is such that it is easy to account for the extra calories.

This goes with all sorts of other psychological characteristics. We find, for example, that obese girls do not apply to go to college even though they may have the marks for it.

This is a pattern of behavior which we do not find in boys. In boys we find that inactivity is probably less of a factor, and that the psychological makeup of the obese boy is quite different from that of the obese girl. Again to give just one example, the matter of applying to college is not correlated with body weight.

It is difficult to know to what extent this has to do with the pressure of society on the obese child, rather than vice versa.

I think this type of environment may be a poor one in which to study the effect of activity on body weight. I was struck, when we made a study in India a few years ago, by the correlation between body weight and prevalence of obesity and physical activity. In that case, however, the range in physical activity of the subjects studied was enormous as compared with the range that we see in our society, where other effects, such as social factors, obscure the differences due to activity.

DR. JAMES SALTER (*Toronto, Ontario, Canada*): There was one factor I wondered about. Most men work for a living whereas this is not quite the case with women. If your data were replotted, would they show that the obese women were the housewives whereas the non-obese women were the ones who went out to work? I wondered whether there was any correlation there at all.

DR. ALBERT STUNKARD: That is a good question but I am afraid I cannot answer it. We only have information on the occupation of ten of the women we studied and the housewives in this group did not walk less.

DR. VINCENT DOLE (*New York, New York*): I wonder whether it is possible to reverse the interpretation. Rather than thinking of underactivity as being etiologic in causing the obesity, is it possible that the state of physical activity is something of a symptom of the nutritional state?

For instance, it is true that normal people who have been starved show quite quickly a drop-off in physical activity. This seems to be quite compensatory as energy supplies dwindle. We do not normally think of the fat person as being undernourished, because to look at him, he is so overfed. However, it is perfectly possible that these people are functionally undernourished, that the actual supply of fuel to their muscles is low.

One would then say that perhaps a critical experiment in this would be to study the physical activity of fat people during and after a course of weight reduction.

It has been my impression—just as a sort of passing impression without a measurement—that physical activity in fat people, whether or not they have an initial depression of physical activity, is likely to become subnormal when these fat people reduce toward their normal weight, even when they are still somewhat obese.

DR. STUNKARD: The idea that obese persons may be undernourished is an interesting one. I had not really thought of that, and will have to think it over.

Actually, we have a great deal of information on what happens to obese people when they reduce. In almost every single instance in which there has been a weight loss physical activity has gone up. We must have thirty or forty such examples. This is quite symmetrical. Which comes first, is pretty hard to say. My hunch is that whatever makes them go on a reducing diet, also makes them walk more, so there may not be any causal connection between those two factors.

Human Energy Expenditure Studies in the National Institute of Arthritis and Metabolic Diseases Metabolic Chamber

I. Interaction of Cold Environment and Specific Dynamic Effect

II. Sleep

E. R. Buskirk, ph.d.,* R. H. Thompson, ph.d.,† R. Moore, ph.d.‡ and G. D. Whedon, m.d.§

One of the primary interests in the Metabolic Chamber program is energy balance. Prior to a discussion of two studies currently underway, a brief description of the chamber is given.

The Metabolic Chamber of the National Institute of Arthritis and Metabolic Diseases, at the National Institutes of Health, is essentially a well controlled air-conditioned room[1] in which the environment may be varied and where a person can remain on a self-sufficient basis for hours or even days (Fig. 1). Metabolic rate and heat loss can be measured continuously while the person remains in the chamber. Metabolic rate is measured indirectly by assessment of oxygen consumption and carbon dioxide production through continuous sampling of expired air. Heat loss is measured with conventional skin thermocouple harnesses and heat exchange discs applied to the surface of the skin. Most of the instrumentation is outside the Chamber proper. An observer can record the activities of the subject under investigation by watching him through the large thermopane window and can communicate either intermittently or continuously with him through a two-way microphone-speaker system. A schematic diagram of the general "plumbing" layout that handles the air stream from the subject is shown in Figure 2.

An open system helmet or "hood" method similar to the one used by Benedict[2] is employed in the indirect calorimetry. The volume of air drawn through the hood around the head of the subject (the expired air is part of this volume) is measured continuously with a large wet-test air meter. Oxygen content of the air stream is measured with a modified Beckman (paramagnetic) F-3 analyzer and carbon dioxide content with a modified Baird (infrared) bolometer plant stream analyzer. Each analyzer functions with an operational sensitivity of about ±0.02 per cent concentration of gas on the scale range most frequently used.

It is difficult to measure total metabolism continuously and accurately over relatively long periods of time. Many of the difficulties experienced by the early and outstanding investigators in the field of calorimetry are still present today. Chief among these difficulties is the diminishing yield relationship between

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Presented at the Brook Lodge Invitational Symposium on Energy Balance, sponsored by The Upjohn Company, September 29, 1959, at Brook Lodge, Augusta, Michigan.

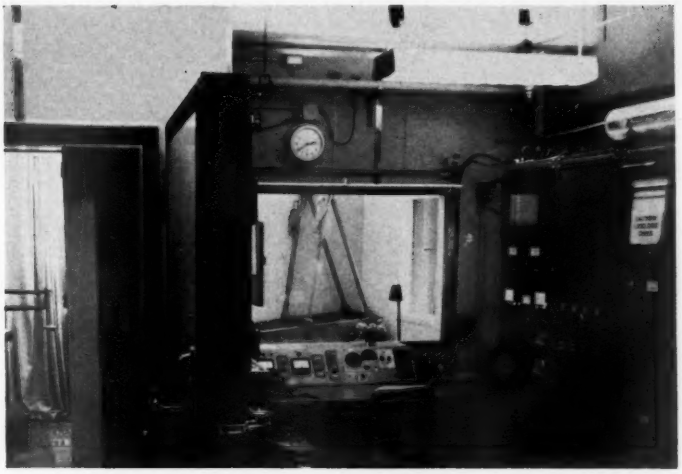

FIG. 1. View of the Chamber from the adjacent laboratory. The double door entry to the Chamber is shown at the left. The treadmill can be seen through the large thermopane window. Chamber equipment controls are located at the desk console. The gas analysis equipment is located in the relay rack at the right. The window in the Chamber wall (to the right) facing the treadmill was planned to give an assuring view of the National Institutes of Health campus.

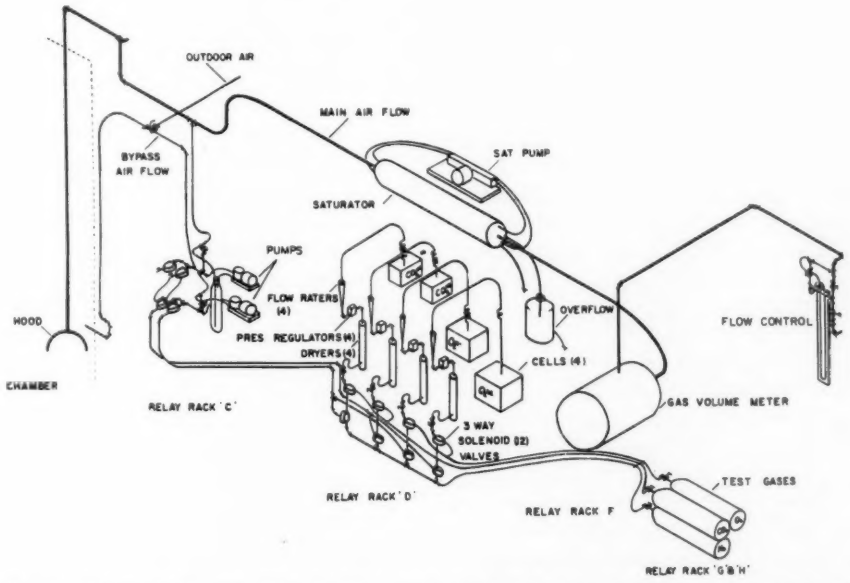

FIG. 2. Schematic diagram of the plumbing required to handle the main air stream from the hood to the vacuum source, and the smaller fraction of the air stream diverted through the gas analyzers. Calibrating arrangement using test gas bottles is also shown.

FIG. 3. A view of the position of the subject and the equipment worn during a typical specific dynamic effect experiment. The subject is consuming a liquid meal through a straw, the glass containing the meal is within an "elephant's trunk" or sleeve designed to facilitate eating without escape of expired air.

the complexity of instrumentation for the purpose of improved sensitivity and continuous, reliable performance. It has been necessary to strive for an "optimal" point of compromise between sensitive but unnecessarily complex instruments and practical utilization and performance.

Two examples of initial attempts at measuring the metabolic rate and exchange of heat in several experimental situations are, (1) measurement of "specific dynamic effect" (SDE) in subjects, who were fed 1,000 kilocalorie meals and exposed to different environmental conditions, and (2) evaluation of changes in daily energy expenditure in persons subjected to weight reduction regimens involving caloric restriction and physical work. Thus far, particular attention has been paid to daily activities such as walking and sleeping.

SPECIFIC DYNAMIC EFFECT (SDE)

Since the time of Rubner's work on dogs,[3] it has been assumed that the elevated heat production after eating is not only useful in maintaining body temperature, but may partially or completely replace thermogenesis normally induced by exposure to cold. The extent of replacement would, of course, depend on the severity of, and the length of time spent in a given environment as well as the quantity and composition of the ingested food. The reason, however, for even partial replacement of one by the other of these two modes of heat production remains somewhat obscure since the locus of heat production may be different for each mode (muscular tissue for shivering and the liver for specific dynamic effect), and the required stimulus for each mode may be different as well. To test the

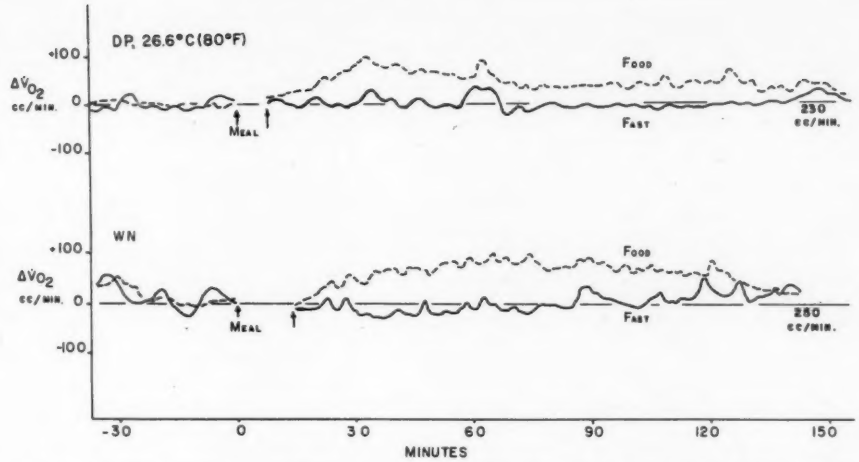

FIG. 4. Specific dynamic effect in two young male subjects who were exposed to 26.6°C. (80°F.) air. The ordinate is the change in oxygen consumption ($\dot{V}O_2\Delta$). A reference $\dot{V}O_2$ value for the zero line is given for each subject. The period between the arrows indicates the time required to consume the meal.

replacement ideas of Rubner as well as to ascertain whether the equipment in the Metabolic Chamber would perform sufficiently well to follow small changes in metabolic rate, the following study was initiated.

Four men, eighteen to twenty-three years of age, clothed in shorts were exposed for three to six hours in various environments ranging from 50° to 80°F. Although a total of forty-two experiments were conducted, the discussion is arbitrarily restricted to representative data from twenty-four experiments in which air temperature was maintained at 50° or 80°F. throughout the course of an experiment. Relative humidity ranged from 30 to 40 per cent, and air movement was less than 50 feet per minute. The subjects rested quietly in a sitting position on a plastic mesh lounge chair. The position and dress of the subject in the chamber are shown in Figure 3. Values for age, height, weight and body density for the four males are given in Table 1A. Values for two female subjects who participated in the sleep study, which will be referred to later, are entered in Table 1B. Experiments were conducted with the subject, (1) fasting throughout the three to six hour exposure, and (2) fed a 1,000 kilocalorie liquid meal (40 per cent of the kilocalories in the form of protein) midway in the experiment.

The curves for oxygen consumption and body temperature, presented in Figures 4,

TABLE 1A
Physical Characteristics of Four Young Male Subjects

Subject	Age	Height (cm.)	Weight (kg.)	Body Density*
D. P.	23	182.2	72.22	1.049
W. N.	19	170.5	63.27	1.071
R. A.	21	183.5	75.56	1.071
J. S.	21	164.5	68.88	1.062

TABLE 1B
Physical Characteristics of Two Female Subjects Including Changes in Body Weight and Density During Experimental Days

Subject	Date	Age	Height (cm.)	Weight (kg.)	Body Density*
K. B.	7/10	18	160.0	70.92	1.021
	7/28			67.74	1.021
	8/19			64.19	1.020
M. L.	7/27	28	162.6	73.81	1.015
	8/4			72.23	1.014
	8/18			69.86	1.017

* Body density was determined with the Siri[4] helium dilution apparatus through the courtesy of N. Berlin of the National Cancer Institute.

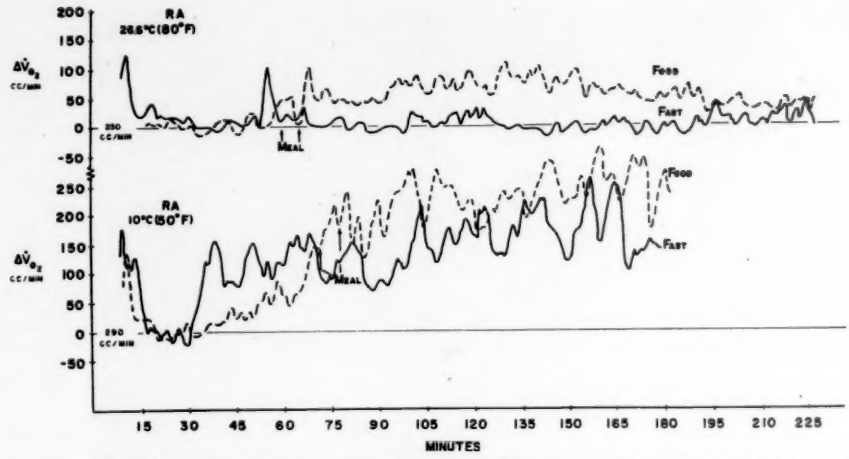

FIG. 5. Comparison of specific dynamic effect at 26.6°C. (80°F.) and 10°C. (50°F.) in subject R. A. Change in oxygen consumption ($\Delta\dot{V}_{O_2}$) is the ordinate. Reference values for the zero $\Delta\dot{V}_{O_2}$ reference line are given.

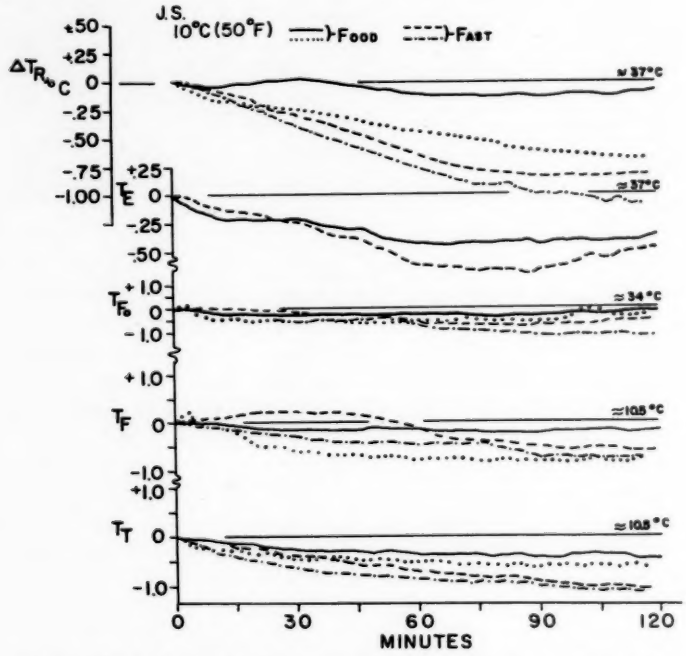

FIG. 6. Change in rectal (ΔT_R), outer ear canal next to the ear drum (ΔT_E), forehead (ΔT_{F_o}), finger (ΔT_F), and toe temperature (ΔT_T) for separate but equivalent periods of exposure of subject J. S. to 10°C. (50°F.) air. Each curve represents a different experiment which serves to illustrate intraindividual temperature differences under fed or fasting condition as well as the effect of food on body temperature. Approximate reference values for the zero lines are provided. Results from the cold exposure period prior to the time the meal was usually fed have been omitted. The considerable cooling that had occurred prior to zero time is not shown.

TABLE II

Elevation in Oxygen Consumption ($\dot{V}_{O_2}$) (cc. per minute) in 80° and 50°F. Air During Periods of Fasting and Feeding

Subject	No.* of Experiments		Fasting† $\dot{V}_{O_2}$ (NF)		Food (F) $\dot{V}_{O_2}$		F-NF $\dot{V}_{O_2}$		$\frac{\dot{V}_{O_2}(\text{F-NF})}{\text{NF}\dot{V}_{O_2}(80°)} \times 100$	
	80°	50°	80°	50°	80°	50°	80°	50°	80°	50°
D. P.	2	2	237	453	281	503	44	50	19	21
W. N.	3	2	258	374	303	445	45	71	17	27
R. A.	2	4	267	410	322	449	55	39	21	15
J. S.	2	4	240	317	283	378	43	61	18	25
Mean	..	..	251	389	297	444	47	55	19	22
Standard	..	..	15	58	19	51	6	14	2	5
Mean Ratio: 50/80	...		1.55		1.49		1.17		...	

* Number of times the subject was exposed to a given environment.
† Values obtained after the identical period of cold exposure as the food (F) values.

5 and 6, are representative curves and not average curves from replicate determinations in a given environment.

OXYGEN CONSUMPTION ($\dot{V}_{O_2}$)

In the comfortable environment (80°F.), the postprandial elevation in $\dot{V}_{O_2}$ was distinct when compared to the fasting curve (Fig. 4). An elevation in $\dot{V}_{O_2}$ could be distinguished within five minutes after eating (essentially no lag). Peak $\dot{V}_{O_2}$ values were usually reached within one hour (about 25 per cent above resting), but frequently the so-called peak more closely resembled a plateau because relatively high values were often maintained for periods of one hour or more. A relatively slow decline in $\dot{V}_{O_2}$ occurred after the plateau period and fasting values were usually reattained within two to two and one-half hours.

The subjects did not remain absolutely quiescent during the pre- or postprandial periods. Movements of the body such as shifting in position were clearly distinguishable as secondary elevations superimposed on the primary $\dot{V}_{O_2}$ curve. Secondary elevations associated with movement were not of sufficient duration and magnitude, however, to alter interpretation of the primary postprandial $\dot{V}_{O_2}$ curve.

At 50°F., the $\dot{V}_{O_2}$ curves were vastly different from the curves obtained in the 80°F. environment (Fig. 5). Shivering was obvious in all records obtained in 50°F. air. Eating had no effect on the shivering mechanism of the body, either in altering the frequency or metabolic intensity of the generalized bouts of shivering. Major $\dot{V}_{O_2}$ peaks, associated with shivering, were discernible with a periodicity of one peak every ten to twenty-five minutes. As in 80°F. air, the postprandial $\dot{V}_{O_2}$ curves were elevated in comparison with the fasting curves. Although the difference between the postprandial and fasting $\dot{V}_{O_2}$ curves disappeared when a bout of shivering in the fasting curve coincided with a quiescent period in the postprandial curve, a distinctly higher $\dot{V}_{O_2}$ was evident during the total one and one-half to two and one-half hour postprandial period.

Table II shows a summary of the oxygen consumption data obtained in the two environments. Results obtained from all four subjects are compared. Each value is the calculated mean (cc. per minute) level after the same duration of both fasting and postprandial exposure to a given environment. The increase in metabolism as a result of exposure to cold was approximately 50 per cent, as represented by the ratios of 1.55 and 1.49 ($\dot{V}_{O_2}$ in 50°F. air divided by $\dot{V}_{O_2}$ in 80°F. air) for the fasting and feeding period, respectively. A postprandial elevation in metabolism was found for all subjects in both environments.

The postprandial effect was independent of environment as indicated by the ratio 1.17 (probably not significantly different from 1.00) obtained by dividing the average incremental increase in metabolism after eating in 50°F. air by the comparable value at 80°F. Thus, cold and eating appear to exert independent effects upon metabolism.

Although Rubner found that the specific dynamic effect replaced the metabolic increment normally associated with exposure to cold in dogs, it appears that in man the metabolic increments associated with eating and exposure to cold produce what resembles "summation." This summation occurred routinely in the four male subjects observed, although there may be individual differences in this response. These experiments with cold exposure correspond to the results obtained by other investigators who noted that the metabolic increment associated with the ingestion of protein was superimposed on the elevated metabolism associated with physical work.[5,6] Another interesting comparison, is that there may be considerable species variation in this response since it has been observed that the critical temperature of rabbits is not lowered if they are fed during exposure to cold.[7]

The concept of simple summation is misleading because it is possible that the heat increment associated with eating may alter the effective stimulus for shivering or change its focus. If eating changes the thermal state of the body, neurogenic stimuli, arising from changing temperatures in the skin (altering peripheral thermal gradients) or in the region of the hypothalamic thermoregulatory centers, could be responsible for the elevation in metabolic rate after eating while exposed to the cold.

To check this possibility, several body temperatures were measured including three skin temperature measurements, rectal temperature, and the temperature next to the ear drum with the outer canal occluded as a "best" measure of hypothalamic temperature. Rectal and ear temperatures were measured with thermistor devices; skin temperatures were measured continuously with thermocouples on two of the men (R. A. and J. S.), and at thirty minute intervals with a dermal radiometer on the other two (W. N. and D. P.).

BODY TEMPERATURE CHANGES ASSOCIATED WITH SPECIFIC DYNAMIC EFFECT

The usual body "core," as represented by rectal temperature (T_R), response to cold (of sufficient intensity and duration to activate the shivering mechanism) is an initial rise in T_R followed by a continuing fall. The initial elevation in T_R is produced by reduction in the size of effective body core by vasodilation which decreases heat loss at the surface of the skin and results in sequestering the heat produced by liver and other active tissues in a smaller volume. After peripheral cooling has progressed, even though active shivering occurs and heat production is raised, the body core begins to cool, by convection and conduction, and the temperature of the body core falls. Sufficient heat is not produced by shivering to maintain core temperature at the level which occurs prior to exposure to cold. This typical pattern was observed during the fasting and preprandial periods in all subjects exposed to cold. Representation of the postprandial phase is provided by Figure 6 in which data obtained from subject J. S. are presented. Although body temperature curves for the other three subjects were not identical to those from J. S., they were similar. Subsequent remarks, therefore, will reflect impressions gained from all temperature records.

During the postprandial phase, the slope of the slowly declining T_R curve was decreased, and the difference in slope from the fasting T_R curves was usually apparent within sixty minutes after eating. The decrease in slope, or relative elevation in T_R after food, continued for the duration of each experiment, and T_R occasionally reached values which equaled or exceeded those obtained when the subject was first placed in the Chamber. The mean for the relative postprandial elevation in T_R, with respect to the mean fasting value was approximately 0.5°C. (0.9°F.) for the four subjects. This relative elevation was no different from that which normally occurred after food eaten in 80°F. air. Ear temperature (T_E) changed in much the same way as rectal

temperature. In 50°F. air, the decrement in internal homeothermy resulting from exposure to cold, was partially ameliorated by postprandial thermogenesis. Hypothermia was not totally relieved, however, since the peripheral tissues remained chilled.

The postprandial changes in forehead (T_{Fo}), finger (T_F), and toe (T_T) temperature in subject J. S. are shown in Figure 6. T_{Fo} changed little in either environment. Considerable variation characterized the interindividual response in T_F and T_T. While T_F and T_T were frequently, but not always, higher sixty minutes or later after eating than when fasting, the differences averaged only 0.5° to 0.6°C. In subjects W. N. and D. P., these differences ranged from 0.5° to 1.5°C. while in subject R. A. no differences were observed. Exposure to cold did not appear to alter the response of peripheral temperature to food in a given person.

Because of the delay in change of body temperature after eating, there is no evidence that the increment in metabolic rate was initiated by a specific change in a body temperature or thermal gradient after food was given in a cold environment. In other words, the metabolic increment did not appear to be induced reflexly. It could, however, have been reinforced by thermal stimuli. For example, comparison of body temperatures with metabolic rates in the same experiment revealed that body temperatures were only elevated after an increase in metabolic rate. Thus, in certain areas in the periphery (fingers and toes) a larger skin-to-air gradient existed after increase in metabolism. This larger gradient, a potential source of neurogenic stimulation to the thermoregulatory center, could have reinforced or sustained the metabolic increment. No evidence of reflex vasodilation, as indicated by an associated rise in skin temperature, after food intake was noted prior to an increase in metabolism. This finding would appear to rule out peripheral neurogenic stimulation as the cause of elevated postprandial metabolism in the cold.*

Since one of the topics discussed at this symposium on energy balance was satiety signals, these metabolic and body temperature results are relevant to the thermal hypothesis produced by Brobeck,[8] whose view is that elevated heat production and the resultant rise in hypothalamic temperature (mediated by warm blood perfusing the brain) provides a probable stimulus (among other stimuli) to the satiety center. Upon receiving the satiety signal the person stops eating to prevent hyperthermia.

While excess heat must be produced during the first few minutes after eating, this fact could not be demonstrated with temperature measurements. Upward deflection of the T_R and T_E curves were observed within the first fifteen minutes after eating on only two occasions. A concomitant rise in T_F or T_T in 80°F. air, or a rise which could be divorced from the Lewis cyclic blood flow phenomenon in 50°F. air, which produces temperature fluctuations, was never observed within fifteen minutes after eating. A thermal satiety signal, if effective, would be manifest within fifteen minutes. These data do not disprove the hypothesis of Brobeck. The almost immediate increase in metabolism after eating may support his view even though associated temperature changes were not demonstrated.†

SLEEP

As recently as 1956 the following statement prepared by Hoffman et al.[9] appeared in the literature: "the measurement during sleep of metabolic rate by means of indirect calorimetry, although desirable, is impossible with the techniques usually available." In these experiments and in others[10,11] where metabolic rate was measured during sleep, the "hood" method

* These summary comments should be qualified, since our temperature measurements were limited with respect to the number of body sites, and in two subjects, the frequency of measurement at these sites. In addition, we did not have temperature measurements in the hypothalamic area other than external ear canal next to the drum, or deep body temperatures other than rectal. Nor do we have peripheral blood flow or peripheral or central nerve impulse data. While our information is suggestive, we cannot unequivocally offer definitive cause and effect information concerning augmented peripheral or central stimulation for cold induced thermogenesis after eating.

† A recent paper by Graf, W. *Acta physiol. scandinav.* (supp.), 46:160, 1959 describes an almost immediate rise in liver temperature following the feeding of 400 gm. protein.

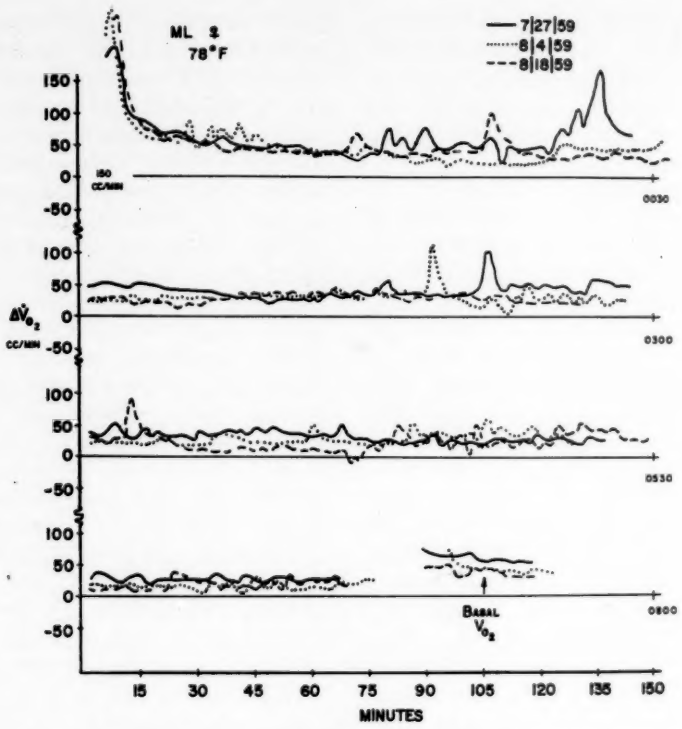

FIG. 7. Change in oxygen consumption ($\Delta\dot{V}_{O_2}$) during the night hours on three different nights for subject M. L. The night was divided into periods of approximately two and one-half hours each by fifteen minute interruptions for instrument calibration. Subject M. L. retired about 10 P.M. each evening and was awakened at approximately 6:30 A.M. each morning. After awakening she arose, was weighed, voided and went back to bed. The instruments were recalibrated and a basal $\dot{V}_{O_2}$ measurement was obtained.

has proved quite satisfactory for measuring oxygen consumption during the night hours.

Interest in sleep stems from an over-all interest in factors responsible for inter- and intraindividual variation in metabolic rate under controlled conditions. For example, during sleep interindividual differences in metabolic rate of 30 per cent or more have sometimes been encountered.[10] In view of these relatively large individual differences and because obese subjects have been shown to be less active physically during the day, two questions may be asked. (1) Are obese subjects also less active during the night hours? (2) Do they rest in bed quietly without moving and thereby expend relatively less energy than their lean counterparts?

Questions such as these have provided motivation for initiation of studies on sleep, and future investigation may lead to the answers.

Thus far, only six people have slept in the Chamber; the results obtained from two of these people, both women, appear in Figures 7 and 8.* The three separate curves presented for each subject were interrupted at hours 0030, 0300, 0530, and before the basal measurements were made. This was done to calibrate the instruments at regular intervals. Advantage was taken of these breaks in the continuous record to divide the night into periods by plotting the data of each night in a single figure. When in the Chamber, each

* See Table 1B for the physical characteristics of the two female subjects.

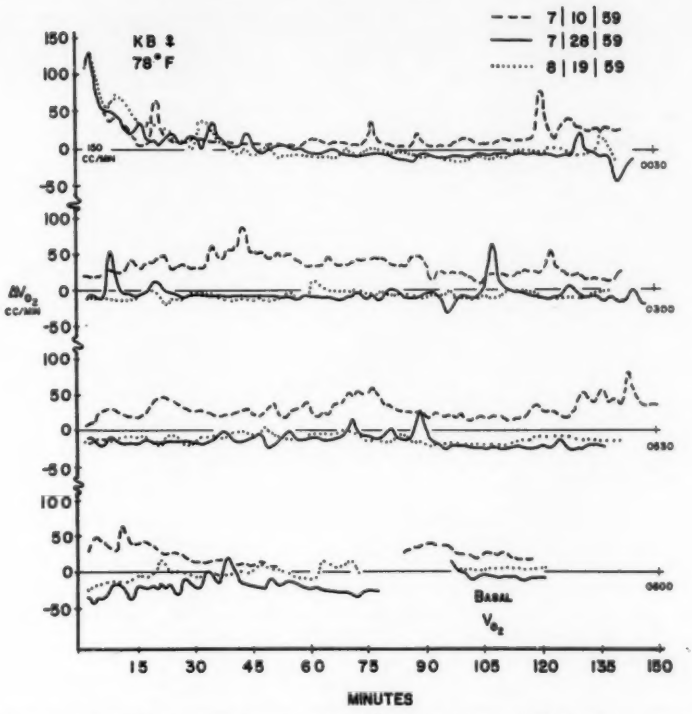

FIG. 8. Change in oxygen consumption ($\dot{V}O_2$) during the night hours on three different nights for subject K. B. For additional legend see Figure 8.

subject slept on a comfortable hospital bed with her head inside the special hood adapted for the sleep studies as demonstrated by the male model in Figure 9.

Inquiry on the morning following a Chamber test revealed that the nights had been restful for both women on all but one occasion. On July 10, 1959, subject K. B. was awake several times and failed to receive what she considered a good night's rest. Several physical activity peaks were evident and a relatively high metabolic level was sustained in comparison with subsequent nights.

Inspection of Figures 7 and 8 revealed that $\dot{V}O_2$ varied considerably during the night hours. Slow changes in metabolism during the night have been shown by previous work[10,12,13]; however, in the curves presented in Figures 7 and 8 both the slow changes (effects of getting into bed, residual SDE, etc.) and acute changes associated with body movement during the night are clearly outlined.

The important applications resulting from analysis of these continuous records relate to the following: first, the relative accuracy of an average value selected to represent night time metabolic rate in twenty-four hour energy expenditure appraisals; and second, the relationship between basal oxygen consumption and oxygen consumption during the hours of the night. It is interesting that the early morning $\dot{V}O_2$ values prior to awakening are usually less than the basal values measured thirty minutes later. In all tests this difference ranged from 0 to 15 per cent of the respective basal value. Differences of this order have been found in other sleep-awake comparisons.[14,15] The immediate and incorrect conclusion, without knowledge of the metabolic picture for the entire night, is that metabolic rate during sleep is 8 to 10 per cent less than basal. It has been commonplace in studies of twenty-four hour energy expenditure to apply an average value of basal minus 8 to 10 per

FIG. 9. Example of the hood employed in the sleep experiments. Subject is free to move his body during the night and may sleep on his back or on either side. Two way communication with the subject is possible whenever desired.

cent to the night period. The results presented in Figures 7 and 8, however, emphasize that if selection of an average value is necessary, a value equal to basal would be more representative. Certain risk is involved in assigning an average value to any night without observation of the physical activity of the subject while in bed, since physical activity is the important variable, effecting changes in the expenditure of energy during the hours of the night. The previous comments are pertinent only if a reasonably precise knowledge of night time energy expenditure is required. The expected intraindividual variation between nights of 50 to 125 kilocalories per eight hours only amounts to about 2 to 5 per cent variation in estimated twenty-four hour expenditure of energy. Variation of this magnitude may be tolerated for many purposes.

With respect to the second consideration mentioned, basal metabolic rate does reflect the metabolic tendency during the night hours. If the metabolic rate is high during the night hours, the basal rate also tends to be high. As pointed out earlier, however, the basal rate does not necessarily and implicitly reflect the metabolic rate during the early morning hours just before awakening.

The nocturnal metabolic patterns were similar for the two women. Other studies in which sampling was performed at intervals indicate that larger interindividual variations do exist.[10] The women walked about eight miles per day, five outdoors and three indoors, on the treadmill (7.5 per cent grade) at a pace of three miles or more per hour. They were physically tired at the end of each day. Since they resided on a metabolic ward, their personal and recreational needs were met adequately. Their daily lives were almost identical and this similarity of daily living was probably responsible for the small interindividual variation observed.

SUMMARY

A brief description and graphic portrayal of the Metabolic Chamber facility and associated instrumentation have been presented.

In a reinvestigation of one phase of the classical work of Rubner[3] on specific dynamic effect, it was found that the increment in heat production associated with eating a 1,000 kilocalorie meal (40 per cent of kilocalories was protein) and the increment associated with exposure to cold showed summation when human subjects were fed while resting in the cold. The two dogs studied by Rubner reacted differently; heat produced after eating meat

simply replaced the production of heat that would normally have occurred with exposure to cold. The dogs apparently shivered less after eating, and their critical temperature was lowered (environmental temperature at which shivering and elevated heat production is first observed). In contrast, no change was observed in the shivering response in our human subjects after they ate in a cold environment.

Changes in metabolism during the night hours were demonstrated by continuous determinations of oxygen consumption on two women who slept in the Chamber. The results suggest that an energy expenditure value equal to basal would be more appropriate to apply in estimates of twenty-four hour energy expenditure than a value of basal minus 8 or 10 per cent. A relationship between the level of oxygen consumption for the night hours and the basal rate was suggested; if night time metabolic rate was high, so also was the basal rate. Although the depth of sleep is frequently greatest during the first hour or two after retiring, a relatively high oxygen consumption was routinely observed during this period. Continuing SDE and repayment of oxygen debt, incurred during the preparation for bed, may be responsible for this observation.

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DISCUSSION

Dr. David Fleming (*Cleveland, Ohio*): This is outside the present study, but did you take electroencephalographic recordings of any of these subjects as they slept to see if there were higher or lower levels of electroencephalographic activity which might correspond to changes in metabolic activity which you did observe?

Dr. Buskirk: No, we did not. We hope to get information of this kind in the future. Dr. Kleitman and the people in his group at the University of Chicago have information of this kind but not in conjunction with metabolic studies. They have data on movement in bed in conjunction with the electroencephalographic records.

Efficiency of Energy Utilization in Farm Animals

MAX KLEIBER, D.SC. *

THE UTILIZATION of energy in farm animals means transfer of energy from feed to animal products or work. Some biochemists use the term utilization in a different way. They measure, for example, utilization of an organic compound by the carbon dioxide which is produced from the compound. If this production of carbon dioxide is paralleled by heat production, as it usually is, then this means waste and not utilization for farm animals.

Efficiency is the ratio of the energy in the desired product to the energy in feed. It may be total efficiency, namely, G/I with G = gain in energy of milk or body substance, or eggs or work, and I = energy in feed; or it may be partial efficiency, namely, $\Delta G/\Delta I$ with ΔG as a change in gain and ΔI the corresponding change in food energy taken in. ΔI may be expressed as heat of combustion of feed, or digestible or metabolizable energy of feed.

PARTIAL EFFICIENCY FOR MAINTENANCE

Armsby[1] measured the partial efficiency of the energy present in timothy hay for maintaining steers. He carried out difference trials, measuring the production of heat (by the heat loss) of steers at two levels of feeding below maintenance in a respiration calorimeter (Table I).

Changing the ration from 10.21 to 6.27 pounds of hay per day decreased the metabolizable energy by 3,776 kilocalories per day. The decrease in heat production amounted to 1,748 kilocalories per day. With the lower ration, the steer lost 2,296 kilocalories chemical energy of body substance more than he lost with the higher ration. Therefore, 4.04 pounds of hay saved 2,028 kilocalories of body substance which amounts to 502 kilocalories of body substance saved per pound of hay. The partial efficiency of the metabolizable energy in timothy hay was the following.

$$\frac{\Delta G}{\Delta I} = \frac{2{,}028}{3{,}776} = 0.54 = 54\%$$

PARTIAL EFFICIENCY FOR FAT PRODUCTION

Difference trials with steers on fattening rations were conducted by Kellner.[2] He measured the carbon and nitrogen balances of the steers at two different levels of food intake above maintenance in a respiration apparatus. Table II gives the results of one of the difference trials in which Kellner measured the utilization of starch for formation of body fat. The experiment is somewhat complicated because the steer gained weight from one trial to the next. This weight gain increased his maintenance requirement and decreased the amount of feed energy available for fat production. By estimating the maintenance requirement Kellner could calculate how much metabolizable energy of the ration was available for fat production.

The addition of starch to the diet increased the metabolizable energy available for fat production from 13.7 to 16.1 kilocalories. This difference in metabolizable energy of 2.4 megacalories produced an increase in fat production of 1.4 megacalories. Thus the

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* Professor.

Presented at the Brook Lodge Invitational Symposium on Energy Balance, sponsored by The Upjohn Company, September 29, 1959, at Brook Lodge, Augusta, Michigan.

TABLE I
Determination of Net Energy Value of Timothy Hay for Maintenance

	Dry Matter of Hay Eaten (pounds)	Metabolizable Energy (kilocalories)	Heat Produced (kilocalories)	Gain of Energy (kilocalories)
Period 4	10.21	9,544	9,812	−268
Period 3	6.17	5,768	8,064	−2,296
Difference	4.04	3,776	1,748	2,028
Difference per pound dry matter of hay	...	935	433	502

TABLE II
Kellner's Measurement of Net Energy in Starch*

	Metabolizable Energy in Feed (megacalories)	Body Weight (kg.)	Metabolizable Energy		Net Energy Body Fat (megacalories)
			Maintenance (megacalories)	Production (megacalories)	
Basic ration	26.8	600	13.1	13.7	7.5
Basic ration	29.9	650	13.8	16.1	8.9
Difference: starch	3.1	50	0.7	2.4	1.4

NOTE: Net availability of starch net/metabol. = 1.4/2.4 = 0.58 = 58%.
* Difference trial. Landw. Vers. Stat., 53, 1,900.

TABLE III
Energy in Nutrients

	Heat of Combustion (Digestible Energy). (kilocalories)	Metabolizable Energy (kilocalories)	Net Energy (kilocalories)	Partial Efficiency of Metabolizable Energy (%)
Digested starch 1 kg.	4,180	3,760	2,360	63
Digested protein 1 kg.	5,780	4,700	2,230	48
Digested fat 1 kg.	8,820	8,820	5,680	64

partial efficiency of starch for fat production was the following.

$$\frac{\Delta G}{\Delta I} = \frac{1.4}{2.4} = 0.58 = 58\%$$

Kellner ran similar difference trials using protein (wheat gluten) and fat (peanut oil) as additions to the basic ration. A summary of the results is shown in Table III.

Kellner assumed that the net energy of a feed could be calculated as the sum of the net energy content in protein, fat and carbohydrate of the feed, but when he added hay to a basic ration its fattening effect was considerably less than that predicted from the net energy in the constituents. Kellner noticed that the discrepancy was greater the higher the crude fiber content in the roughage became. On the average, 1 gm. crude fiber depressed the fattening effect 1.36 kilocalories, the net energy in 0.58 gm. of starch.

PARTIAL EFFICIENCY FOR WORK

Horses and mules still do some farm work. As early as 1888 Emil Wolff[8] measured their efficiency by feeding horses combinations of

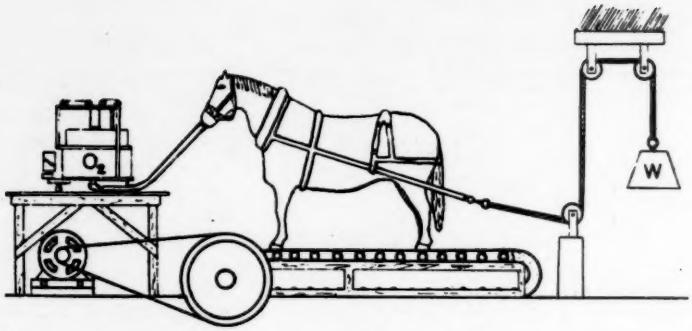


FIG. 1. Measuring efficiency of working horse. (From: BRODY, S. and CUNNINGHAM, R. *Missouri Research Bull.*, 38:238, 1936.[5])

hay and oats, adjusting their daily work so that on any given ration the horses maintained their body weight.

These were experiments of long duration. At the turn of the century (1898) Zuntz and Hagemann[4] investigated the work efficiency of tracheotomized horses walking on a treadmill against a measured pull. This arrangement, using a face mask instead of a tracheal cannula, is shown in Figure 1 (taken from a publication by Brody and Cunningham[5]). The picture is self explanatory.

One of the results of Zuntz and Hagemann[4] is shown in Table IV.

During rest, the horse consumed 1.68 liters oxygen per minute. This corresponded to a heat production of 8.3 kilocalories per minute. When the horse performed 5,313 meter-kilograms per minute, equivalent to 12.5 kilocalories of work per minute, the catabolic rate rose to 53.4 kilocalories per minute. Thus, 12.5 kilocalories of work was produced by an increase of catabolism of 45.1 kilocalories. The partial efficiency of body energy for work was

$$\frac{\Delta \text{ work}}{\Delta \text{ catabolism}}, \text{ was therefore } \frac{12.5}{45.1} = 0.28 = 28\%.$$

This is an efficiency of net energy for work because the body energy, such as fat, glycogen, protein and even glucose in the bloodstream, has already resulted from a transfer of food to body substance with the heat increment involved.

TABLE IV
Measurement of Partial Efficiency of Body Energy for Work in Horses

	Oxygen Consumption Per Minute (liters)	Catabolism Per Minute (kilocalories)
Rest	1.68	8.3
Work*	10.80	53.4
Increase by work	9.12	45.1

NOTE: Partial efficiency of body energy kilocalories of work/kilocalories of catabolism = 12.5/45.1 = 0.28 = 28%.

* 5,313 meter-kilograms/minute = 12.5 kilocalories/minute.

PARTIAL EFFICIENCY FOR MILK PRODUCTION AND GROWTH

In contrast to work and fat production, milk production and growth are not mainly energy transformations. This is especially

TABLE V
Energy Balance Per Day*

	Ingested (kilocalories)	Excreted (kilocalories)
Food†	35,150	10,110 (feces)
Digested	25,040	1,380 (urine)
		2,770 (methane 291 l.)
Metabolizable	20,890	6,060 (milk 8.5 kg.)
Catabolizable	14,830	16,170 (heat)
Gain in body substance	1,340	

* *Cow 1007.* Weight 460 kg., weight$^{3/4}$ = 99 kg.$^{3/4}$ February 19 to March 2, 1940.

† *Food: Dry matter.* 5,258 gm. Sudan hay, 519 gm. beet pulp, 521 gm. casein, 2,110 gm. glucose.

TABLE VI
Efficiency of Food Utilization vs. Protein—Ratio in Rations of Baby Chicks

Ration	Protein Ratio* (%)	Energy Intake Per Day Per Chick (kilocalories)	Total Efficiency	
			N (%)	Energy (%)
4.5 Casein 40.5 glucose 55 basal mix	36	123	35	20
20.0 Casein 19.0 glucose 55 basal mix	49	135	22	11
32.5 Casein 9.5 glucose 55 basal mix	64	126	16	9

* Protein ratio = $\frac{\text{Energy in protein}}{\text{Energy in total food}}$ 100.

true for the role of protein in these types of animal production. In the production of work or fat, protein can be regarded as a fuel. It can replace or can be replaced by fat or carbohydrate as sources of chemical energy. For the formation of casein or body protein, however, feed protein is a source of amino acids—building material instead of fuel.

Milk production or growth is not as closely correlated to feed intake as is fat production or work, at least within a normal range. Milk production is more dependent on other factors than food. Therefore, for milk production or growth, difference trials are not suitable for measuring efficiency.

Efficiency for comparison can still be measured, for example, by measuring the carbon and nitrogen balance and (for partial efficiency), estimating a given maintenance requirement without milk production or a catabolism without food.

The measurement of carbon and nitrogen balance in a lactating cow led to the results in terms of energy as shown in Table v. The Table illustrates the difference between the metabolizable and the catabolizable energy for lactation. This difference is especially obvious for the utilization of protein. A lack of clarity about this difference has led to confusing terms such as "corrected metabolizable energy."

One gm. of digestible protein may have 6 kilocalories metabolizable energy if it is converted to casein or body protein, but it has only 5 kilocalories catabolizable energy because in catabolism in the animal, about 20 per cent of the chemical energy of protein is lost as chemical energy of urine.

The mean partial efficiency for milk production can be calculated by subtracting the maintenance requirement from the metabolizable food energy or adding the basal metabolic rate to the energy in the daily milk, and then dividing by the metabolizable energy in the ration. This gives a ratio of total net energy (milk + maintenance) to total metabolizable food energy.

From the results given in Table v, total efficiency can be calculated.

Milk energy per day	6,060	kilocalories
Gain in body (this is a loss)	−1,340	kilocalories
Production effect of food	4,720	kilocalories
Metabolizable food energy	20,890	kilocalories
Total efficiency	$\frac{4,720}{20,890}$ 100 = 24%	

Estimate of mean partial efficiency:

Production effect of food	4,720	kilocalories
Basal metabolism estimated $70 \cdot W^{3/4}$	6,930	kilocalories
Production + maintenance effect of food	11,650	kilocalories
Mean partial efficiency of metabolizable energy	$\frac{11,650}{20,890}$ 100 = 56%	

EFFECT OF PROTEIN TO ENERGY RATIO ON EFFICIENCY FOR GROWTH OF CHICKS

That efficiency for growth, like that for milk production, depends on the protein to energy ratio of the ration, illustrated in Table VI. In this case the partial efficiency

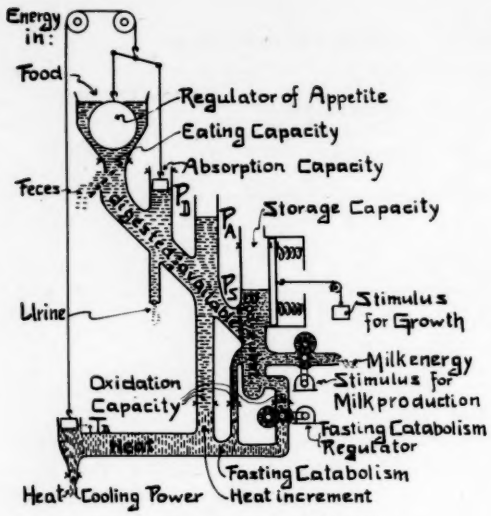


FIG. 2. Regulation of food intake.

decreases as the protein content of the ration increases. Again, what is measured is not partial efficiency because the trial is not a difference trial.

TOTAL EFFICIENCY AND FEED CAPACITY

The total efficiency is the ratio of gain over cost, or energy in product over food energy.

$$e_{tot} = \frac{G}{I}$$

G = energy in grain

I = energy in food

If the partial efficiency is independent of the plane of nutrition then the gain is proportional to that part of the food energy which is available for production. This relation may be formulated as follows:

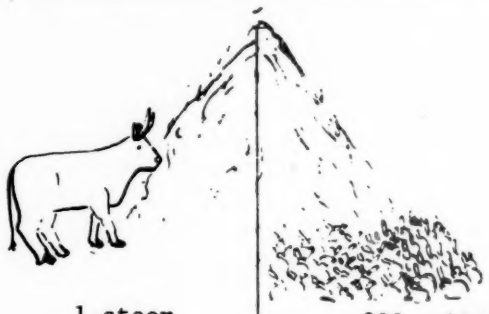
$$G = e_p(I - M)$$

where

e_p = partial efficiency
M = food energy required for maintenance G and I as defined above.

From that expression we can get the formulation of total efficiency as follows.

$$e_{tot} = \frac{G}{I} = \frac{e_p(I - M)}{I} = e_p - \frac{e_p \cdot M}{I}$$



Animals	1 steer	300 rabbits
Body weight total	1,300 lb.	1,300 lb.
Food consumption per day	16 2/3 lb.	66 2/3 lb.
1 ton of food lasts:	120 days	30 days
Heat loss per day	20,000 kcal.	80,000 kcal.
Gain in weight per day	2 lb.	8 lb.
Gain from 1 ton of food	240 lb.	240 lb.

FIG. 3. Food utilization versus body size.

TABLE VII
Utilization of Sun Energy for Animal Products

	Total Efficiency*		
	N/U (%)	U/S (%)	N/S (%)
Milk			
1,200 lb. cow fed hay and beets			
20 lb. milk per day			
35 per cent partial efficiency	16	0.26	0.042
Pork			
quick fattening 40 to 220 lb. in twenty weeks			
potatoes, concentrates and silage	22	0.07	0.015
Eggs			
50 eggs per 100 hens per day			
10 Skandinavian feed units	4	0.05	0.002

* N = energy in animal product available for man; U = energy in animal feed; S = radiant energy from sun.

The net energy for maintenance is $e_p \cdot M$, which is the amount of body energy saved by the maintenance food. This is the energy lost without food or the basal heat production. Thus, the equation is the following.

$$e_{tot} = e_p - \frac{B}{I}$$

The total efficiency of food utilization is greater the smaller the ratio, basalmet abolism/food intake, or the greater is the reciprocal, food intake/basal metabolism, which in an animal fed as much as it will eat may be called relative food capacity.

Food intake according to Adolph[6] is one of the best regulated animal functions.

Figure 2 shows an early, perhaps premature, attempt to present the interaction of various conditions affecting food intake.[7] The scheme is an attempt to integrate two major theories on regulation of food intake, the chemostatic[8] and the thermostatic[9] control of food intake.

FOOD UTILIZATION AND BODY SIZE

The total efficiency of animals can be expressed as the difference between the partial efficiency and the ratio of basal metabolic rate to rate of food intake (the reciprocal of relative food intake).

TABLE VIII
Area Yielding Food Energy for One Man Per Year*

	Efficiency (%)	Area Required	
		Square Meter	Acres
Algae (Warburg)	50	1	0.002
Potatoes	0.10	600	0.15
Grain	0.05	1,200	0.30
Prunes	0.04	1,500	0.37
Milk	0.04	1,500	0.37
Pork	0.015	4,000	1.0
Eggs	0.002	30,000	7.4

*Requirement: 10^9 calories per man year.
Flux density of sun's radiation in California: 1.6×10^9 calories per square meter per year.

The partial efficiency is independent of the size of body as is the relative food capacity. Chicks and steers take in on the average from four to five times as much food energy as they lose from their body when they fast.[10]

We can therefore conclude that body size as such does not affect the efficiency of food utilization. Rabbits can be as efficient food utilizers as steers. Biologists know that the heat production per unit of body weight of small animals is greater than that of large animals. It appears, therefore, that small animals should waste more food energy per unit of time and therefore large animals should be more efficient.

The first part of the argument is correct but the conclusion is wrong as illustrated in Figure 3.

One ton of hay is fed to a steer weighing 1,300 pounds, and another ton of the same hay to 300 rabbits whose combined weight is also 1,300 pounds. The steer eats $16^2/_3$ pounds of hay per day, the rabbits $66^2/_3$ pounds of hay. Therefore, the ton of hay lasts 120 days for the steer and only thirty days for the rabbits. As expected from the law of body size and metabolic rate, the 300 rabbits produce daily four times as much heat as the steer. But the rabbits gain nearly four times as much body substance per day as did the steer. Therefore, the gain per ton of hay is the same

for the steer (in 120 days) as for the rabbits in thirty days. The only difference is duration.

UTILIZATION OF SUN ENERGY FOR ANIMAL PRODUCTS

Table VII gives an estimate of the utilization of sun energy for milk, pork or egg production. The table is based on the estimated total efficiency of conversion of food energy to energy of the animal product. The next estimate is the energetic efficiency of plant production involved. In each case, the product of the two efficiencies is the total efficiency of conversion of sun energy to the energy of the animal product.

Table VIII gives an estimate of the area required to supply the food energy for one man for one year when he is satisfied with algae or depends on eggs alone for food.

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DISCUSSION

DR. HERVEY (*Sheffield, England*): I remember some work that was done in England during the war, when there was great enthusiasm for producing food, and people used to keep rabbits as part of their drive to win the war. One person kept some rabbits, and wore a Douglas bag during the time he was tending these rabbits. He compared the energy consumed in the extra work of looking after the rabbits with the energy which could be derived from eating them at the end of the process. He found that he used up about twice as much energy tending them as he received from them at the end of the period.

Does Dr. Kleiber know whether the steer is any better from that point of view than the three hundred rabbits?

DR. KLEIBER: Was this person a farmer?

DR. HERVEY: He was not.

DR. KLEIBER: He would have even a worse time with a steer.

Premigratory Hyperphagia in Birds

EUGENE P. ODUM, PH.D.*

THE ability of migratory birds to store and utilize large amounts of fat has been known for a long time but only recently been investigated systematically. The rapidity of deposition and utilization, the abruptness of the inception of the temporary obesity, and the precision of seasonal timing raise many questions regarding energy balances, environmental triggering mechanisms and internal physiologic mechanisms. Recently it has been shown that migratory obesity in some species can be induced in the laboratory by long photoperiods, making possible experimental as well as analytical study. The unusual lipid metabolism of migratory birds becomes of interest in connection with the basic problems underlying obesity in man.

These investigations began ten years ago when a study of weights of banded white-throated sparrows wintering on the university campus revealed two distinct peaks in weight, one in mid-winter and one just before northward migration.[1] The actual amounts and body distribution of lipids determined in a subsequent study showed that the premigratory deposition, or "migratory fat," differed in amount and body distribution from "winter fat."[2] "Migratory fat" was deposited in all major regions of the body, especially intraperitoneally in the abdomen. "Winter fat" deposits were smaller and largely subcutaneous with little fat present in peritoneal regions Premigratory deposition in nature in the spring was rapid, ten days or less being required for an increase in total lipids from about 6 per cent to 15 to 25 per cent of live weight.

All major organs of the body showed an increase in fat deposits with the exception of the heart which remained low in fat content at all times. Total liver lipids increased from about 6 per cent (wet weight of the organ) to 10.6 per cent during the premigratory deposition period. Liver phospholipids showed little seasonal change. Two premigratory birds, however, had high values suggesting that brief periods of high phospholipid content may be associated with the rapid mobilization of fat.

The type of lipid deposition induced experimentally out of season by long photoperiods was identical to the "migratory fat" found in premigratory birds under natural conditions.[3] Wolfson,[4–7] deBont,[8] Farner and Mewaldt[9] and King and Farner[10] have demonstrated that increasing the length of the day at any time following the termination of a post-migratory refractory period will produce lipid deposition in migratory species of fringillids (finches), but not in non-migratory species of the same family. Farner[11] and Wolfson[12] also have reviewed the role of photoperiodism in the regulation of spring migration.

The birds which have been intensively studied (notably species of the genera Zonotrichia, Junco and Fringilla) are relatively short range migrants which winter in temperate regions. Maximum deposition of fat in these species is 25 to 30 per cent of total live (wet) weight. Odum and Connell[13] and Odum[14] have demonstrated that long range or overseas migrants, which cross the Gulf of Mexico nonstop from the United States to Yucatan or Central and South America accumulate larger

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TABLE 1
Three Patterns of Migratory Obesity in Birds

Species and Condition	Number of Birds	Total Lipids (gm. per 100 gm.)					
		Live (Wet) Weight		Total Dry Weight		Non-fat Dry Weight	
		Average	Maximum	Average	Maximum	Average	Maximum
*1. Short Range Migrants, Build-up During Migration**							
Savannah sparrow							
Spring premigration level (April)	34	9	17	24	42	33	73
Level near end of fall migration (October to November)	14	20	26	43	53	77	114
Experimentally induced by long photoperiods	28	23	34	46	63	98	170
2. Short Range Migrants, Build-up During Migration†							
White-throated sparrow							
Spring premigration level (April)	19	17	25	40	50	68	110
Experimentally induced by long photoperiods	22	16	24	38	48	66	106
3. Long Range Migrants‡							
Pretrans-Gulf flight level in scarlet tanagers (October)	29	43	52	68	73	216	276
Premigration level in hummingbirds (September)	4	43	46	76	78	313	350

* Attain moderate obesity but begin migration before peak deposition (i.e., Passerculus sandwichensis).[17]
† Attain moderate obesity but begin migrating after peak deposition (i.e., Zonotrichia albicollis).[2,3]
‡ Become extremely obese prior to long non-stop flights (i.e., Pirangia rubia and Archilochus colubris).[13,14]

amounts of fat just prior to the long flights. Birds killed at airport ceilometers or television towers while migrating southward through the southern United States proved to be extremely obese, with up to 50 per cent of the wet weight or 75 per cent of the dry weight of the body composed of fat.

TYPES OF MIGRATORY OBESITY IN WILD BIRDS

There are at least three patterns of lipid deposition in migratory birds. Examples of each type are shown in Table 1 where average and maximum total ether extracts are listed as percentages of the total wet weight, dry weight and non-fat dry weight of the body. In the first type, birds becomes restless (i.e., develop what is known as "migratory unrest" or "zugunruhe") and take short flights before there is much build-up in the reserves of fat. As the season progresses the lipid deposition rate increases making possible longer flights. Thus, savannah sparrows leaving their wintering grounds at the latitude of Augusta, Georgia have small reserves of fat while many birds striking a television tower at the same locality on the southward return trip are moderately fat (Table 1). A pattern of this type is probably frequent in early spring migrants wintering in the United States. The triggering of the migratory urge before maximum fat deposition takes place is of adaptive value since low reserves of energy at the start would prevent long flights which might move the bird into unfavorable early spring weather conditions.

In the second type of pattern, illustrated by the white-throated sparrow, moderate obesity develops on the wintering grounds. Migratory unrest does not develop until after maximum fat deposition is achieved; presumedly birds are then able to make longer flights at the start of the migratory journey. A similar situation holds for the closely related white-crowned sparrow (Z. leucophrys) which is being studied by Farner and his students on the west coast.[15] This second type of pattern is common in late spring migrants.

As shown in Table 1 fat deposition induced in the laboratory by long photoperiods in both the white-throated and the savannah sparrow is similar in magnitude to peak levels observed in nature. In these species experimentally induced obesity is no greater than the temporary migratory obesity which occurs in nature. When captive birds of one of the species of migratory finch are subjected to long days in December or January (past the post-migratory refractory period but well before normal premigratory deposition) no increase in weight occurs for several weeks, then fat deposition begins suddenly. In other words, there is a lag between the start of the environmental stimulus and the abrupt inception of fat deposition; within certain limits the longer the length of day the shorter the lag time. In no case, however, is this period less than twenty days.[6,16] Once fat deposition has begun, maximum deposition in the white-throat (a "pattern 2" species) is reached in about ten days (as is the case in nature). In contrast, Connell[17] found that three or four weeks was required for the same build-up in the savannah sparrow (a "pattern 1" species) under the same conditions of light. Little is known about triggering mechanisms for fat deposition in autumn premigratory birds which comes during periods of decreasing day lengths. It is not known if light or other environmental factors are involved or if an internal "clock" mechanism (perhaps preset by the long days of spring) comes into play.

The third pattern of migratory obesity (Table 1) is characteristic of long range migrants which breed in North America but winter in the tropics. Scarlet tanagers and ruby-throated hummingbirds are typical of a group of small land birds which are trans-Gulf migrants, and fly non-stop over at least 600 miles of open water (Fig. 1). The data on tanagers shown in Table 1 are from a group of birds which struck a television tower on the Florida Gulf Coast on October 5, 1956. All birds in this group were extremely obese; presumedly they were just beginning the southward flight over the Gulf. The obese hummingbirds were taken from a premigratory aggregation near Augusta, Georgia, in late September just a day or two before all the hummingbirds migrated from the aggregation. In mid-September a large number of hummingbirds gathered in a patch of Crotalaria flowers. As described by Norris, Connell and Johnston[18] the birds gorged themselves with nectar, rapidly increased in weight and achieved the level of fat deposition shown in Table 1 just before departure.

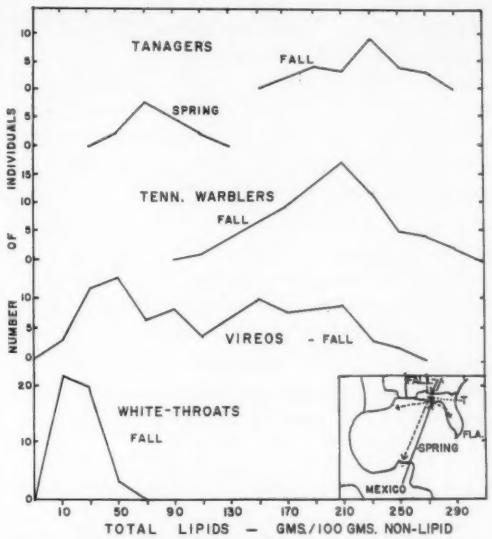

FIG. 1. Frequency distribution of lipid levels in samples of birds killed at a Florida Gulf Coast television tower. Insert shows location of the tower and spring and fall migration routes of migratory birds which breed in the United States or Canada and winter in the tropics.

From the standpoint of the per cent of live weight the tanagers are the fattest (up to 52 per cent), but in terms of per cent of non-fat dry weight the hummingbirds rank higher (up to

350 per cent). Thus, the hummingbird whose non-fat dry weight is about 0.7 gm., or whose fat-free wet weight is about 2.6 gm., may carry up to 2.6 gm. of fat (equal to about 23 calories) at the peak of premigratory deposition. It has already been shown that the available energy from such a depot is more than enough for a flight of 600 miles.[13] Based on the estimate that regardless of size of warm-blooded animals the energy requirements for sustained hard work is three times that required for "existence metabolism" (a rate which is known for small birds), the maximum fat depositions achieved by tanagers and other similar overseas migrants make possible non-stop flights of up to 1,500 miles.[14]

TELEVISION TOWER INVESTIGATIONS

An opportunity to obtain data on lipid levels of different types of migrants at various stages of migration was provided in 1956, when Mr. Herbert L. Stoddard began his studies of bird mortality at the Gulf coast television tower. Mr. Stoddard visited the tower at dawn during the spring and fall migratory periods for several years. To facilitate collection of specimens, 35 acres of ground under the tower were cleared, sodded and treated with insecticide to reduce the attack of ants and other insects on the fallen birds. When large numbers of birds were killed Stoddard mapped their position so that it was possible to infer the direction of flight. Large samples of birds of some 100 species have been stored in deep freeze and are available for studies on lipid deposition. Frequency distribution by fat classes are shown in Figure 1 to illustrate some of the contrasting patterns.

The data on the fall scarlet tanagers are the same as that listed in Table I. Other species which have proved to be extremely obese in the fall include the summer tanager (P. rubra), bobolink (Dolichonyx oryzivorus), several species of warblers (Parulidae) and thrushes (Turdidae). Tanagers found in the spring were not as fat (second frequency curve, Fig. 1) since presumedly they had just crossed the Gulf from Yucatan or other points to the south. The spring birds still had appreciable fat reserves bearing out the assumption that long-range migrants accumulate greater deposits of fat than are usually needed. Gulf coast bird observers have demonstrated that many trans-Gulf migrants in spring do not stop at the first land fall, but continue inland for many miles. Only during bad weather do the birds seek the first land, and thus are forced low enough to strike the tower.

As shown in Figure 1, other species striking the tower in the fall exhibit different patterns from those of the tanagers or Tennessee warblers. Frequency distribution of lipids in red-eyed vireos (Vireo virens) is bimodal. This species uses two migration routes, one over the Gulf and one via Florida and the West Indies. It might be speculated that the fat peak represents birds which would have gone by sea and the lean peak birds which would have gone by land. Since the tower is located near the southern limit of the winter range of the white-throated sparrow (which migrates no further south than the Gulf coast) it was interesting to find that the birds were uniformly lean. The frequency distribution shown is similar to that of a non-migratory species during the same season. One of the white-throated sparrows in this sample was the leanest individual bird yet encountered; its total lipids were less than 0.5 gm. or about 2 per cent of wet weight, a level lower than that of non-migrating birds. Thus migrating birds can and do use up all but a small amount of the fat present in the body.

ENERGY BALANCE

Table II summarizes the balances of energy between food intake and fat production in caged savannah sparrows subjected to a steady fifteen hour day and to the normal increasing photoperiods of early spring at a time when birds would normally start migrating northward. In these experiments birds were captured in the field and measurements of their food intake and weights made at four day intervals. The three week predeposition period included an adjustment period during which time some birds lost weight and then regained it, followed by approximately two weeks when no net increase in weight occurred. The deposition period was the period when a net increase in weight occurred. As has already been dem-

TABLE II

Fat Production in Relation to Metabolized Food Intake in Two Experiments with Savannah Sparrows

Experimental Period	Length of Period Days	Metabolized Food Intake (calories/gm./day)		Fat Deposition	
		Total	Available for Fat	Calories/ gm./Day	Percent Available Energy
1. Fifteen Hour Photoperiod Beginning February 16th (Twenty-Eight Birds)					
Predeposition period	25	0.74	...	0	..
Deposition period	26	0.83	0.09	0.042	47
2. Normal Photoperiod Beginning February 28th (Thirteen Birds)					
Predeposition period	27	0.76	...	...	..
Deposition period	35	0.83	0.07	0.035	50

Metabolized food equals caloric value of food eaten minus caloric value of excreta. Calories of food and fat are listed in terms of fat-free wet weight (not total weight) of birds. Average fat-free weight of birds in experiment 1 was 15.9 gm. and in experiment 2, 16.1 gm. Air temperatures were maintained relatively constant (±5°F.).[17]

onstrated the fat-free weight does not change appreciably during the deposition period; hence the fat-free weight (determined for each bird at the termination of the experiment) subtracted from the live weight provides a good estimate of the fat content of the bird at the time of weighing. As may be seen from Table II, the daily rate of fat deposition was greater in birds subjected to longer photoperiods, but metabolized food intake increased in both groups in the same proportion. Thus, in both groups of birds about 50 per cent of the energy intake over and above that of the predeposition period was converted into fat. Also, additional energy was dissipated in the activity associated with the "migratory unrest." Since fat deposition reduces energy needed for regulation of temperature it is possible that energy available for fat and activity was greater than indicated in Table II. In nature, rising spring temperatures would increase available energy so that only a moderate increase in food consumption would be needed.

Fat production during the deposition period is not uniform throughout the period. A fairly steady but moderate increase occurs for a time followed by a sharp increase in rate during the last few days of the deposition period. Therefore, average figures shown in Table II do not show the rapid deposition which occurs at the end of the experimental period, or just before migration in type two and three birds in nature. Rates of fat production during the week of most rapid deposition for selected individual caged sparrows and wild hummingbirds are shown in Table III. The sparrows include those birds showing the best response to long photoperiods. Data on the hummingbirds are not based on changes in the same birds, but on the increase in weight which occurred in the population during the last week of the premigratory aggregation described earlier in this paper. Since food intake was measured for groups of sparrows and not individuals the intake for the individual birds on which Table III is based is not known. However, the energy converted into fat in these birds was greater than the average increase in food intake by the experimental group as a whole (Table II). It would appear from the data in Table III that the rate of fat production in long-range migrants may be three times greater than the peak rates achieved by short range species, which is not unexpected since the former are able to accumulate more fat in a shorter time than the latter.

COMMENTS

One of the unanswered questions is whether

TABLE III

Comparison of Rate of Fat Production During the Five to Eight Day Period of Most Rapid Deposition in Caged Savannah Sparrows and Wild Hummingbirds

Species and Conditions	Number of Birds	Fat Production Rate (calories/gm./day*)	
		Average	Maximum
Savannah sparrows subjected to long photoperiods in the laboratory	6	0.17	0.24
Hummingbirds premigratory aggregation in nature	7	0.46	0.72

* Calories are in terms of fat-free weight which averaged 15.9 gm. for sparrows and 2.65 gm. for hummingbirds.[17]

migratory obesity is the result of regulatory changes in energy balance, i.e., the "hyperphagic-hypothalamic" type,[19] or the result of changes in hormone balance, i.e., the "metabolic" type, or a combination of both. Experiments show that birds become hyperphagic more or less in proportion to the magnitude of lipid deposition. The over-all increase in food intake was 12 per cent (Table II), but during the week of maximum deposition the increase was about 22 per cent. In the white-throated sparrow, which exhibits a more rapid build-up of fat than the savannah sparrow, food consumption increased to 50 per cent.[3]

As already indicated there are no data on the increase in food intake by long-range migrants. The rapid build-up and even more rapid utilization of fat by long range migrants suggests that something more than simple hyperphagia is involved. So rapid is lipogenesis and lipolysis in these species that the peak amounts of fat are easily missed unless specimens are examined at exactly the right time. A working but unproved hypothesis is that migratory obesity involves the temporary alteration of both nervous (hypothalamic) and metabolic pathways. It might also be postulated that the migratory fat body is a temporary organ with its own enzyme systems which promote rapid lipogenesis and lipolysis.[20] In view of the ineffectiveness of exercise in reducing obesity in man and rats the ability of birds to use up large amounts of fat in a twelve to forty-eight hour migratory flight is indeed remarkable. One difference is evident between migratory obesity and the hereditary metabolic type obesity of certain strains of mice. In the latter the fat-free weight may be markedly altered (other tissues reduced in favor of adipose tissue), while in migratory birds the fat-free weight remains largely unchanged, although the glycogen content may be reduced in place of lipids. A migratory bird could not function if the muscles or other tissues vital to flight were sacrificed to make room for fuel.

Some of the investigations planned for the future are the following: (1) Attempting to induce migratory obesity in a long range migrant species in the laboratory. To date, no one has worked with such species in captivity and these birds may make important experimental animals since they have "abilities" in regard to lipid metabolism not possessed by ordinary laboratory animals or man. Elucidation of mechanisms may well come from experiments with such species. (2) Continue study of deposition patterns of birds from the television towers. (3) Investigate, in detail, the chemical and histologic nature of migratory fat. (4) Continue studies on energy balance with attention to the utilization as well as the production phase and experiment with restricted food intake to determine if deposition of fat can occur without hyperphagia.

SUMMARY

The unusual lipid metabolism of migratory birds is of interest in connection with human problems of obesity for two reasons: (1) lipogenesis and lipolysis are extremely rapid in some species and (2) temporary obesity can be readily induced experimentally by photoperiod manipulation.

Wild birds appear to exhibit three patterns of migratory obesity: (1) short range migrants which attain moderate obesity but begin migration before peak deposition; (2) short range migrants which begin migration after peak deposition; (3) and long range migrants which become extremely obese (50 per cent of wet weight, 75 per cent or total dry weight or

300 per cent of non-fat dry weight of body composed of fats) just prior to long flights (such as over the Gulf of Mexico). Energy for the long flights is derived entirely from lipids. Experimental work so far has largely concerned short range migrants in which moderate hyperphagia and lipid deposition can be induced by direct stimulation of long photoperiods. New data obtained from birds killed during migration at television towers indicate that experimental work with long range species will prove instructive. A working but unproved hypothesis is that migratory obesity involves a temporary simultaneous alteration of both nervous pathways (i.e., hyperphagic-hypothalamic obesity) and metabolic pathways (i.e., metabolic type of obesity).

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DISCUSSION

Dr. C. N. H. Long (*New Haven, Connecticut*): This is a field of study of fat metabolism that is completely new to me. Have you gotten to the point of putting hypothalamic lesions in a hummingbird yet, Dr. Odum?

Dr. Odum: We are excited about getting some of these long-range migrants into captivity. The humming bird is one that you can keep in captivity and feed on sugar and water with a little protein supplement. The bobolink is another good prospect for experiments.

I believe that these birds have an ability that rats and people do not have in that they undergo this rapid deposition and loss of fat.

We want to investigate the utilization of fat. It is fantastic that a bird of 20 gm. fat-free weight can utilize 20 gm. of fat in a flight, say, of twenty-four or thirty-six hours. This would be unheard of by any human being. I understand it takes months and years to reduce an obese person to normal. These birds return to normal within two or three days.

Dr. Theodore Van Itallie: (*New York, New York*): Do you have any idea what happens to lean body mass during these migrations when they are using their fat stores?

Human beings who are subjected to periods of fast will use 85 per cent of their calories in the form of fat, but they will break down a great deal of protein.

A test of how efficiently these birds use their fat would be to find out how much nitrogen deposit occurs simultaneously.

DR. ODUM: There is a little indirect evidence from birds striking the TV tower as they are finishing their migration. We have carefully compared the fat-free weight, which would be the lean weight, with that of those birds which are fat, and find it slightly but significantly less. In other words, in this long migration they not only lose fat, but they also lose a small amount of other materials.

This is the only factor which affects fat-free weight. Sex does not affect fat-free weight nor does age if you take into consideration the different sizes of the birds. But season does. In other words, the birds that have finished migration are lighter.

However, they will not use up all the fat. Even experimental birds, that lost 5 gm. of fat-free weight, still had 0.5 gm. of fat. So in all our calculations we assume, of course, there is 0.5 gm. that cannot be used.

We have one or two wild birds which are down to 1 or 1.5 per cent but have seemed perfectly healthy otherwise.

DR. JAY TEPPERMAN (*Syracuse, New York*): Can these animals be made to refrain from increasing their food intake by exposing them to minimal amounts of light during times when they might be expected to increase it?

DR. DONALD S. FARNER (*Pullman, Washington*): Specifically with respect to the behavior of fat deposition without change in daily photo period, this does not happen. If you keep birds continuously on short days, they do not put on weight. There is no innate annual function in this, at least in the species on which I work.

This hyperphagia that comes prior to spring migration is a real light-stimulated hyperphagia. There is no question that this is a direct stimulation by light, not just more time, because of more daylight, to feed.

Indirectly but, I think, quite soundly it must operate down at least to the level of the hypothalamus. We do not have lesions. I cannot argue quite that directly.

We should also point out that we have a few scraps of evidence as to what is going on here. Simultaneously with this hyperphagia, there are some interesting changes in metabolism and in the little bit of endocrine picture that I can see.

It appears that during this period there is a reduction, by atrophy, of alpha islet tissue. Along with this, there is a marked reduction in blood sugar levels: from something like 450 to 500 mg. per cent down to 250 mg. per cent, which is low in birds.

We think that perhaps we are dealing with a reduction in glucagon output. This is consistent with the fact that pancreatectomy in most birds, instead of producing a hyperglycemia, produces a hypoglycemia, which would seem to indicate that normally glucagon rather than insulin is in the control from the pancreas.

I hope somebody here can give me a clue as to what this might mean. As soon as this fat deposition begins to reduce, there is a marked reduction in glycogen storage, both in liver and in skeletal muscle. To give you an idea of how completely dependent these birds then become on fat (white-crowned sparrows), when a bird is ready to start migrating in the evening, he has enough glycogen to keep him going, if he sits still, for somewhere between three and five minutes, and after that it has to come out of fat.

DR. SALIH J. WAKIL (*Durham, North Carolina*): Have you prepared any enzyme preparations from the liver of these birds before or just prior to their migration, to see if there is any noticeable difference in fatty acid synthesis or fat synthesis?

DR. ODUM: No. The only thing I know about the liver is that total lipids, and in some cases phospholipids, increase during premigratory deposition.

INCIDENT SPEAKER: Do you believe the same thing may happen, say, in locusts?

DR. ODUM: Insects?

SPEAKER: Yes.

DR. ODUM: Yes, by all means. A student just finished a thesis in which he compared fat deposition in field mice, locusts, and savannah sparrows. They have the same, almost identical, sort of pattern. The ones that hibernate have a modest fat deposition. Those that migrate have a large fat deposition. Monarch butterflies as well as other migratory insects show the same thing.

DR. J. A. F. STEVENSON (*London, Ontario, Canada*): If you prevent the hyperphagia, at least the hyperphagia taking effect, by restricting the food available, rather than interfering with the natural change in the length of day, does the bird then refuse to migrate? Does he know he is not fat?

DR. ODUM: Dr. Farner and I want to do this experiment but we have not as yet.

DR. FARNER: This is obviously the experiment that no one seems to have done, but there is a bit of evidence along this line.

I should point out that fat deposition as a result of increased photo periods is only one of a whole series of changes, which included gonadal development, molt and so forth.

In spring this series is in a definite sequence. But when we use artificially elongated photoperiods in winter, we sometimes disrupt the sequence, so that the bird puts on fat before he molts.

I do have some records of birds that in winter, under artificially long days, first showed Zugunruhe, that is, nocturnal activity, and than later put on fat. So from this shred of evidence we might reason that these are actually two separate things that ordinarily are timed, fat first and then migration, but that nevertheless basically are caused separately and that migration is not a direct cause of the fat deposition. This is awfully slim, but it is all we have to offer.

DR. ODUM: I think in the comparison of the savannah and white-throated sparrows we had just accidentally showed this; that is, one species migrates late and

puts on its complete fat before it migrates, whereas the other one starts to move, gets restless, etc., which cause it to start to migrate, before the fat is completed. I presume this bird travels just as far as it can with the fat and then must stop and recuperate its stores of energy before moving along further.

The evidence is that a bird slowly moving northward in the spring perhaps increases his deposition of fat as he gets closer to his nesting ground, so that the last hop may be the longest whereas the first one was the shortest. The overseas migrants cannot do this.

Birds migrate at night mostly. They go to sleep, just as your people in that chamber, but they will wake up suddenly, become restless, and then begin to migrate if they are ready. Otherwise they sleep all night. This is known as night unrest.

The birds that go overseas do not get restless until their fat deposits are complete: in other words, until they get the 50 or 45 per cent. And then they go overnight.

These other birds, on the other hand, that are adapted to migrating over land, have a gradual build up of fat.

Dr. Farner shows the Zugunruhe beginning in his species toward the end, in this case, of the fat build-up.

Our evidence from the field is that this restlessness begins much before this. Each species has its own pattern. This is adaptive. You can only understand this thing by putting it in nature, because it must adapt to the conditions. This is why you get some different answers when you approach it from the standpoint of what the organism must be to survive in this situation.

Another thing we have determined are the limitations that the food supply places on them. Of course, we can now calculate accurately the total requirement of food of the bird and where they obtain this. Some of them can get it in the north; some of them, of course, have to go further south in order to find food.

DR. LILLIAN RECANT (*St. Louis, Missouri*): Can you induce hyperphagia in non-migratory birds by any light stimuli?

DR. ODUM: Definitely not.

One of the most interesting examples of that is a species which Dr. Farner and others have worked on, which has races within the species. In some cases they are a non-migratory race and a migratory race. If you put both of these under the stimulus of the photoperiod, the non-migratory race does not respond at all. Its gonads respond, and there are other responses, but not fat deposition. So there is no doubt that these are separate mechanisms.

The Mechanism of Fatty Acid Synthesis

SALIH J. WAKIL, PH.D.*

As early as 1907, Raper,[1] recognizing the fact that almost all the naturally occurring fatty acids are composed of an even number of carbon atoms, had suggested that these acids are produced by the condensation of some highly reactive substance containing two carbon atoms. The experimental evidence was first provided by Smedley-MacLean and Hoffert[2] who reported that there was an appreciable increase in the fat and sterol content of actively metabolizing yeast cells when incubated in an aerated medium containing various two-carbon compounds, such as acetic acid and ethyl alcohol. With the use of isotope and tracer technics, Sonderhoff and Thomas[3] confirmed these observations by demonstrating that deuterium-labeled acetic acid can be incorporated into fatty acids and sterols by yeast cells. Later, Rittenberg and Bloch[4,5] found, upon feeding $CD_3C^{13}OOH$ to mice, that the isolated fatty acids were labeled with both deuterium and C^{13}, indicating that both carbons of the acetate are used for the synthesis of fatty acids. They concluded that successive condensation of acetate had taken place to form the long-chain fatty acids.

Although acetate was implicated in the synthesis of fatty acids, the active form in which it is used was not known, and it was assumed to be equivalent to the "active acetate" required for the acetylation reactions. The discovery of Coenzyme A (CoA†)[6,7] and the elucidation of its structure by Lipmann and his collaborators[8] was a notable achievement and a milestone in our understanding of the nature of the acetylation reactions, fatty acid oxidation and many others. Thus, active acetate was identified as AcCoA,[9] which was found to be the end product of oxidation of fatty acids by soluble enzyme system.[10–14]

Stadtman and Barker[14–20] were the first to demonstrate the conversion of labeled acetate into short-chain fatty acids by soluble enzyme preparations from Clostridium kluyveri and to implicate a role for CoA in the synthesis fatty acids. Klein and Lipman[21,22] reconfirmed this observation and extended it to the synthesis of sterols and fatty acids in yeasts and in rats. This information, together with data on the incorporation of isotopic acetate into fatty acids, was sufficient to close the gap between oxidation and synthesis of fatty acids, which were thought to be alternate aspects of a single reversible process. This view was further strengthened when the various enzymes involved in the sequence of fatty acid oxidation were shown to be reversible.[10,11] Indeed, Professor Lynen[23] began his Harvey Lecture with the sentence, "Let us consider the fatty acid synthesis," and then proceeded to present the results of his work on the various enzymes of the β-oxidation sequence and to conclude that "the β-oxidation of fatty acids proposed by Knoop is nothing else but the reversal of this cyclic process."

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† The abbreviations used in this paper are the following: CoA or CoAHS, Coenzyme A; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; PPi, inorganic pyrophosphate; DPN^+, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPN^+, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; AcCoA, acetyl CoA.

This concept of the synthesis of fatty acids was generally accepted by the biochemical community until recently when two new discoveries changed our thinking. The first discovery was made by Langdon,[24] who was able to reduce crotonyl CoA with TPNH in the presence of an enzyme found in the soluble extracts of the rat liver. The other discovery was that a new enzyme system was isolated at the Enzyme Institute[25] from avian livers which were free from the key enzymes of the β-oxidation cycle. This new system converts AcCoA to long-chain fatty acids in the presence of ATP, Mn^{++} Co_2 and TPNH.

This system is not located in the mitochondria nor in the microsomes but it appears to be associated with particles of smaller sizes than the microsomes, for the whole system may be separated into pellets by centrifugation at 140,000 x g for two hours. Thus the name "non-mitochondrial system," or the "malonyl CoA pathway," has been proposed for this system of fatty acid synthesis, in contrast to a newer system located in the mitochondria, which converts AcCoA to stearic acid in the presence of ATP, DPNH, TPNH and a heat-stable factor.* The latter is called the "mitochondrial system" for fatty acid synthesis. The relationship between the mitochondrial system and the reconstituted system of β-oxidation enzyme[26–33] must await further experimentation, although some common intermediates and enzymes may be involved.

MITOCHONDRIAL SYSTEM FOR THE SYNTHESIS OF FATTY ACIDS

Leloir and Munoz[34] were the first to study the oxidation of fatty acids by a cell-free system consisting of a suspension of washed particles prepared from guinea-pig liver. These particles were later identified as mitochondria by Kennedy and Lehninger,[26,27] and by Schneider and Potter.[35] Them itochondria was shown to contain all the required components for the oxidation of fatty acids provided that "a sparker" in the form of a di- or tri-carboxylic acid was added[36] and that the activated fatty acid was oxidized completely to CO_2 and H_2O by the mitochondria. Furthermore, the mitochondria were prepared on a large scale and were used as a source for the preparation of the soluble enzymes for the oxidation of fatty acids.[30] The various enzymes involved in the fatty acid oxidation sequence have been prepared in a highly purified state[10,11] and each step has been shown to be reversible.

Mii and Green[37] were able to oxidize fatty acids completely to AcCoA in the presence of the purified enzymes. Stansly and Beinert[38] attempted to convert AcCoA to higher fatty acyl derivatives of CoA in the presence of DPNH, a reduced dye and the purified enzymes of the fatty acid oxidation cycle. They were not able to demonstrate any significant formation of a fatty acyl CoA of longer carbon chain than that of butyryl CoA. Their results suggested that the problem of fatty acid synthesis was not merely one of simple reversal of fatty acid oxidation.

In 1955, Langdon[39] discovered an enzyme in the soluble extracts of rat liver that catalyzes the reduction of crotonyl CoA by TPNH. He called this enzyme TPNH-ethylene reductase. Later Seubert et al.[31] were able to isolate this enzyme from mitochondria of pig liver and to purify it extensively by differential centrifugation in the presence of cholate. The purified particulate enzyme was free from the various enzymes of the β-oxidation cycle and it had a wide range of specificity from crotonyl CoA to α-β–unsaturated stearyl CoA. The localization of this enzyme in the mitochondria is of extreme interest in regard to our knowledge of the cellular distribution of the fatty acid synthesizing systems. With the aid of this enzyme, Seubert, Greull and Lynen[31] were able to reconstitute the fatty acid synthesizing system from the purified enzyme of the β-oxidation cycle, thiolase,[40–42] enoyl hydrase[43,44] and β-hydroxyacyl dehydrogenase[40,45] a source of DPNH (alcohol dehydrogenase) and a source of TPNH (glucose-6–phosphate dehydrogenase). With this system they were able to synthesize predominantly octanoic, capric and lauric acids and traces of myristic, palmitic and

* Observations of this system have not yet been published.

TABLE I
Incorporation of C^{14}-Acetyl CoA into Fatty Acids by Mitochondria

Experiments*	Amount Incorporated (mμM)
Complete system†	2.3
Minus ATP	0.0
Minus Mn^{++}	3.8
Minus DPNH	0.0
Minus TPNH	1.4
Plus HCO_3^-‡	2.0
Minus AcCoA; plus malonyl CoA§	2.2
Minus AcCoA and ATP; plus malonyl CoA	0.4

* In each experiment the mixture was incubated at 38°c. for thirty minutes.

† The complete system contains 0.1 μM of C^{14}-AcCoA (60,000 c.p.m. total activity), 2 μM of ATP, 0.5 μM of Mn^{++}, 0.25 μM of DPNH, 0.25 μM of TPNH, 50 μM of potassium phosphate buffer (pH 6.5), 2.2 mg. rat liver mitochondria and water to a final volume of 1.0 ml.

‡ Four μM $KHCO_3$ were used.

§ Thirty mμM malonyl CoA ($HOOCCH_2C^{14}OCaA$, 70,000 c.p.m.) were used.

stearic acids from hexanoyl CoA and acetyl-1–C^{14} CoA.

We have demonstrated that intact mitochondria isolated from pigeon or rat livers can carry on the synthesis of fatty acids from AcCoA (Table I). Mitochondria were prepared in either 0.25 M or 0.88 M sucrose according to known procedures. When the mitochondria were incubated anaerobically with AcCoA, ATP, DPNH, and TPNH, long-chain fatty acids were isolated. The conversion of AcCoA to fatty acids was completely dependent upon the presence of ATP. In the presence of 2 mμM of ATP, 10 to 20 per cent of the AcCoA was converted to fatty acids. The addition of HCO_3^- did not affect the synthesis, in contrast to the non-mitochondrial system which is absolutely HCO_3^- dependent.[25] Furthermore, C^{14} malonyl CoA was only incorporated into fatty acids when ATP was present (Table I): malonyl CoA is known to be decarboxylated to AcCoA by a mitochondrial enzyme[46] and it seems possible that this accounts for the utilization of malonyl CoA in the presence of ATP. The major components of the synthesized fatty acids were stearic acid (40 per cent) with palmitate (29 per cent), myristate (20 per cent) and laurate (20 per cent) constituting the remainder. This is in contrast to the non-mitochondrial system where palmitic acid is the only product of the reaction.

These observations suggest that the mitochondrial system is concerned with the synthesis of long-chain fatty acids, primarily those of C_{18} and C_{20} acids by the successive additions of AcCoA to short-chain acids (C_{16} or shorter acids). These findings are in accordance with the overwhelming information in the literature on this subject.

NON-MITOCHONDRIAL SYSTEM FOR FATTY ACID SYNTHESIS

A decade ago Gurin and his co-workers[46–48] reported the synthesis of long-chain fatty acids from acetate, first in homogenates and later in particle-free extracts prepared from pigeon liver. They found that a water extract of mitochondria is required in addition to the particle-free supernatant fluid in order to convert acetate to fatty acids. This system incorporated labeled acetate predominately into fatty acids rather than into glycerides. The addition of citrate to this system stimulates the process as does Mg^{++} and DPN^+. Acetyl CoA, however, was less efficient as a precursor of the synthesis of fatty acids than was acetate. On treatment of the extract with charcoal[48] it was possible to demonstrate a requirement for ATP, DPN^+ and CoA.

The pigeon-liver system of Gurin and his collaborators was the basis for our extensive work on the mechanism of the synthesis of fatty acids.[49,50] This system can be prepared from the livers of pigeons chickens and rats and also from the kidney of the rat. When any of these tissues were homogenized in the Potter-Elvehjem homogenizer in 0.1 M phosphate, 0.25 M sucrose or 0.88 M surcrose and the mitochondria, microsomes and the soluble fractions were prepared;[10] the soluble fraction

invariably contained all the enzymes required for the conversion of acetate or AcCoA to fatty acids (Table II). The 100,000 x g supernatant fraction shows higher activity per mg. of protein than the whole homogenate. This is due to the removal of the inactive particles (mitochondria and microsomes) from the mixture. The results tabulated in Table II also indicate that the addition of the mitochondria or the microsomes would decrease the activity of the supernatant fluid. The same results were obtained when the tissues were homogenized in the hypertonic sucrose solution (0.88 M) which minimizes the destruction of the mitrochondria during the homogenization. Furthermore, the type of the fitting (loose or tight) of the homogenizer appears to have very little effect on the extraction of the fatty acid synthesizing system from these tissues.

From these experiments it is concluded that the enzymes of the synthesis of fatty acids are localized in the soluble cytoplasmic portion of the cells of the pigeon and rat livers. A similar distribution of the fatty acid synthesizing system was found by Brady et al. in pigeon liver,[51] by Langdon[24] in rat liver and by Popják and Tietz[52,53] in the lactating mammary gland. For this reason we would like to refer to this system as the non-mitochondrial system to differentiate it from the mitochondrial system discussed previously. Klein[54] studied the fatty acid synthesizing system from yeast (Saccharomyces cervisiae) and reported that the conversion of acetate to long-chain fatty acids required both the soluble fraction plus what he called "small particle fraction" (precipitated between 25,000 and 60,000 x g). These particles were free from cytochromes and cytochrome oxidase. This also supports our conclusion that the fatty acid synthesizing system is a non-mitochondrial system in origin.

The fatty acid synthesizing system can be separated from the 100,000 x g supernatant extract by further centrifugation of this extract at 140,000 x g for two to four hours.[25] The resulting supernatant fluid is inactive while the pellets, which were red, contained all the enzymes for the conversion of acetate to fatty acids (Table II). The relationship of the various enzymes to the fatty acid synthesizing sequence in this cluster is not as yet understood and must await further experimentation.

TABLE II
Incorporation of Labeled Acetate with Fatty Acid by Pigeon Liver Fractions

	C^{14}-Acetate Incorporated/hr./mg. of Protein (mμM)
Mitochondria	2.0
Microsomes	0.8
Clear supernatant	18.0
Mitochondria plus microsomes	1.2
Mitochondria plus clear supernatant	5.0
Microsomes plus clear supernatant	8.0
Pellets	42.0

NOTE: The various fractions were prepared by homogenizing pigeon liver in 0.25 M sucrose with the Potter-Elvehjem homogenizer. The whole homogenation was centrifuged at 600 x g to remove the nuclei and the debris of the cell. The mitochondria and the microsomes were isolated by the usual centrifugal fractionation at 9,000 x g and 100,000 x g, respectively. The resulting clear supernatant solution was used. The pellets were obtained by further centrifuging the clear supernatant solution for four hours at 100,000 x g. Each reaction mixture contains 5.0 μM of acetate-1-C^{14} (300,000 c.p.m. total activity), 50 μM of potassium phosphate buffer (pH 6.5), 4 μM of ATP, 0.3 μM of Mn^{++}, 0.1 μM of TPN^{+}, 8 μM of isocitrate, 0.04 μM of CoA, 6.0 μM of cysteine and 10 μM of $KHCO_3$. The final volume was 0.5 ml. and the reaction was started by the addition of 2.5 mg. of protein of each fraction where indicated. The reaction mixture was incubated at 38°c. for two hours.

Purification of the Non-Mitochondrial Enzyme System

The soluble extracts of pigeon liver were fractionated with ammonium sulfate into two fractions by Wakil et al.[49] The first fraction (designated as R_1) was precipitated between 0 and 25 per cent saturation of ammonium sulfate and the second fraction (designated as R_2) was precipitated between 25 to 40 per cent saturation. Both of these fractions were required for the conversion of AcCoA to fatty acids. In earlier studies,[25,50] a third fraction (R_4), precipitated between 50 and 65 per cent saturation of ammonium sulfate, was required

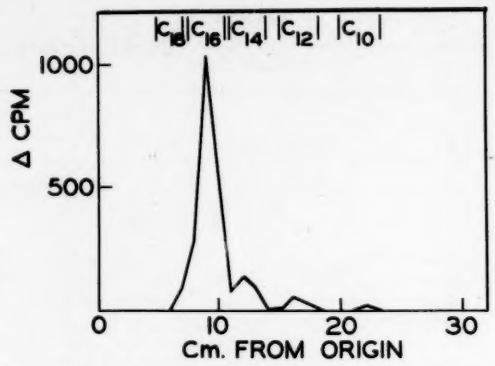

FIG. 1. Distribution of synthesized long-chain fatty acids in the paper chromatographic system of Kaufmann and Nitsch. (From: KAUFMANN, H. and NITSCH, W. H. *Fette U. Seifen*, 56: 154, 1954.[66])

in addition to the fractions R_1 and R_2. This extra fraction R_4 was shown to contain acetate thiokinase and was eliminated when AcCoA was used as the starting substrate instead of acetate.

The two fractions R_1 and R_2 were further purified separately by adsorption on calcium phosphate gel and subsequent elution with phosphate buffer.[25,49] Further purification of these fractions was achieved by chromatography on a cellulose column[32] according to the general procedure of Sober and Peterson.[55] The final fractions obtained are referred to as R_{1gc} (derived from R_1 fraction) and R_{2gc} (derived from R_2). The over-all purification of the fatty acid synthesizing system was more than 100 times above the original 100,000 x g supernatant extract. This is a minimum value since it is difficult to give an exact value in a multi-enzyme system of this nature.

Fractions R_{1gc} and R_{2gc} are free from the various enzymes of the β-oxidation cycle (hyptanoic thiokinase,[39] acetic thiokinase,[56,57] butyryl dehydrogenase,[58] palmityl dehydrogenase,[59,60] the electron transferring factor[61] and thiolase[40,42]). They contain, however, traces of enoyl hydrase[43,44] and β-hydroxyacyl dehydrogenase.[40,45] The TPNH α-β-unsaturated acyl reducing enzyme (discovered by Langdon[24]) which is a key enzyme in the synthesis of fatty acids by the reversal of the β-oxidation sequence,[31] is absent from R_{1gc} and R_{2gc}. These findings further differentiate the non-mitochondrial system from the mitochondrial system in addition to the aforementioned cellular distributions of these two systems.

Products of the Reactions

Considerable experimental evidence has been obtained which supports the concept that fatty acids are synthesized by successive head-to-tail condensations of two carbon units (acetate).[5,62] Much of this information has originated from studies in which whole animals or slices of tissue were used. Brady and Gurin[63] have separated the long-chain fatty acids ($>C_{10}$) that were synthesized by the soluble preparations of pigeon liver and, on decarboxylation of the fatty acids (synthesized from acetate-1-C^{14}), they found the radioactivity in the terminal carbon atom of the fatty acids to be only slightly above the expected value on the basis of successive condensations of acetate units. Popják and Tietz[52,53] separated the products of fatty acid synthesis in mammary gland homogenates and identified them as predominately long-chain fatty acids (C_{10} to C_{18}). Similar results were achieved by Klein,[54] in his work on the fatty acid synthesizing system from yeast, and by Stumpf and his co-workers, who used extracts of avocados.[64]

The products from the conversion of acetate to fatty acids by the highly purified fractions of avian livers (R_{1gc} and R_{2gc}) have been separated and identified by a variety of technics.[65] Figure 1 illustrates the distribution of the radioactivity among the various acids (C_{12} to C_{16}) when they are separated by reverse-phase chromatography.[66] The main peak (>80 per cent) is palmitic acid with traces of myristic and lauric acids. Short-chain acids (C_4 to C_8) do not accumulate under these conditions.

Palmitic acid synthesized from C^{14} 1-acetate was isolated by dilution with unlabeled palmitic acids and was recrystallized from various solvents to a constant specific activity. The resulting acid was decarboxylated by the Schmidt reaction as described by Phares;[67] the liberated CO_2 contained twice the amount of radioactivity of each carbon of the original

TABLE III
Components of the Fatty Acid Synthesizing System of Pigeon Liver

Experiments*	Amount of AcCoA Incorporated (μM)
Complete system†	0.394
Minus ATP	0.000
Minus TPNH	0.000
Minus Mn^{++}	0.030
Minus HCO_3	0.010
Minus R_{1g} (or R_{2g})	0.000

* In each experiment the mixture was incubated for thirty minutes at 38°c.

† The complete system contained 50 μM histidine buffer (pH 6.5); 2 μM ATP; 0.6 μM $MnCl_2$; 0.6 μM AcCoA; 0.8 μM TPNH; 8 μM $KHCO_3$; 1 mg. of R_{1g}; and 0.8 mg. R_{2g}. The final volume was 1 ml.

palmitic acid which indicates a *de novo* synthesis of palmitic acid from AcCoA.

Components and Properties of the Non-Mitochrondrial System

Gurin and his coworkers[47,48] demonstrated that the synthesis of long-chain fatty acids from acetate by the soluble extract, from the liver of the pigeon, is markedly stimulated by Mg^{++} and citrate. When the extract was treated with charcoal, the synthesis was stimulated by the addition of CoA, ATP and DPN^+ in the presence of Mg^{++} and citrate. However, after dialysis of the extract the activity declined almost completely and could not be restored by the addition of the aforementioned cofactors. Porter et al.[50] confirmed these observations. In their earlier reports, the investigators at the Enzyme Institute reported that the following cofactors were required for the conversion of acetate to fatty acids by the reconstituted crude system: ATP, CoA, GSH, DPN^+, glucose-1-phosphate, isocitrate, TPN^+, lipoic acid, Mg^{++} and Mn^{++}. Some of these factors became unnecessary as extensive purification of the enzyme system was carried on, while new components emerged.

The highly purified system of Wakil and Gibson (R_{1gc} and R_{2gc}) has the following requirements for optimal conversion of AcCoA to long-chain fatty acids: ATP, Mn^{++}, HCO_3^- and TPNH (Table III). Little or no synthesis takes place in the absence of any one of these factors. The extra components required in the earlier crude system, such as glucose-1-phosphate and isocitrate, may have been sources for both CO_2 and reduced pyridine nucleotides. Furthermore, isocitrate may play another role in stimulating fatty acid synthesis which is similar to that of other di- and tricarboxylic acids such as malonate, α-ketoglutarate, succinate and fumarate citrate.[50,51,53,63] These polycarboxylic acids stimulate the synthesis without being incorporated partly or wholly into the products.[25] The exact role of these acids is not clear as yet.

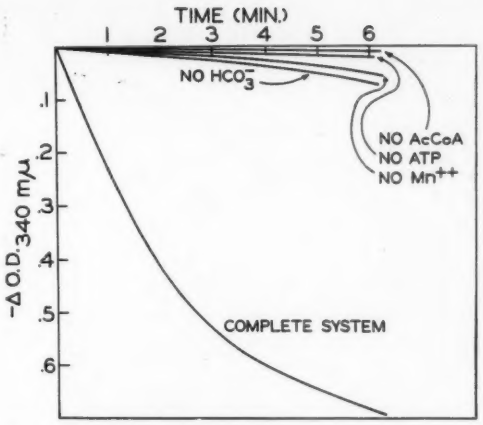

FIG. 2. Oxidation of TPNH. Each cuvette contained the following reagents (except when omitted as indicated): 25 μM of potassium phosphate buffer (pH 6.5), 1.0 μM of ATP, 0.3 μM of $MnCl_2$, 4.0 μM of $KHCO_3$, 0.05 μM of (1-C^{14}) AcCoA and 0.08 μM of TPNH in a final volume of 0.50 ml. The reaction was started by the addition of 0.7 mg. of R_{1gc}. The temperature was maintained at 38°c. At the end of five minutes, 0.032 μM of AcCoA was incorporated into fatty acids.

Earlier assays of the synthesis of fatty acids from acetate were based exclusively on the use of radioisotopes. In the purified enzyme system, however, an equally sensitive and far easier assay method was developed for the measurement of the extent of fatty acid synthesis.[68] This method depends upon the rate of oxidation of TPNH by the fatty acid synthesizing system as measured spectrophotometrically. Figure 2 shows that the

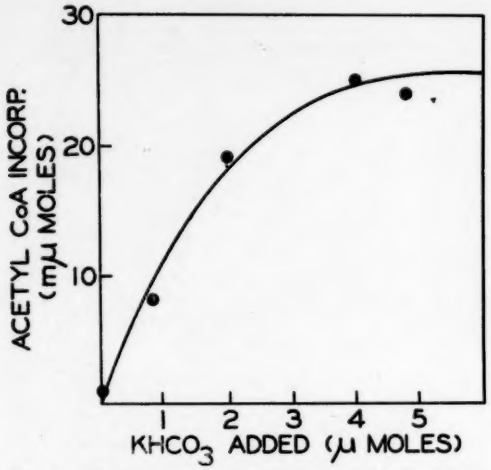

FIG. 3. Each experimental tube contained in a final volume of 0.50 ml. the following reagents: 50 μM of potassium phosphate buffer (pH 6.5), 1.0 μM of ATP, 0.3 μM of $MnCl_2$, 0.08 μM of TPNH and 50 mμM of acetyl-1-C^{14} CoA. The reaction was started by addition of 0.7 mg. R_{1g} and 0.4 mg. R_{2g}. All samples were incubated for five minutes at 38°c. At a bicarbonate concentration of $8\text{-}10^{-3}$ M, the rate of incorporation of AcCoA is equivalent to 0.3 μM/mg. of enzyme/hour.

rate of oxidation of TPNH is strictly dependent upon the presence of all the components of the complete system. The rate of oxidation of TPNH runs parallel with the rate of incorporation of C^{14}-1-acetyl CoA into the long-chain fatty acids as determined by the isolation of the fatty acids from the reaction mixture.[68]

DPNH can substitute for TPNH in fatty acid synthesis, but the rate of oxidation of DPNH is slower than that of TPNH.[68] This cannot be interpreted to be due to the presence of DPNH:TPN^+ transhydrogenase,[69] for under such conditions we could not assay for the transhydrogenase in the two enzyme preparations. This lack of specificity for the pyridine nucleotides distinguishes the non-mitochondrial system from the mitochondrial system[31] in which both TPNH and DPNH are required for the synthesis of fatty acids from AcCoA and short-chain fatty acyl CoA (butyryl CoA, hexanoyl CoA).

The requirement of bicarbonate for fatty acid synthesis was first revealed by the highly purified enzyme preparations of Gibson, Titchener and Wakil.[25] They have shown that this requirement is absolute for all stages of purification and that it applies equally well to the synthesis of fatty acids not only in the avian liver preparation but also in crude preparations from rat liver and rat kidney. Figure 3 illustrates the effect of increasing concentration of bicarbonate on the synthesis of fatty acids in the purified system.

$HC^{14}O_3^-$ is not incorporated into the fatty acids during active synthesis from unlabeled AcCoA (Table IV). This is not surprising, since it has been observed in studies of degradation that acetate or AcCoA is the sole source of the carbon chain of the fatty acid synthesized by this system. For this reason a "catalytic" role for HCO_3^- has been proposed.[25]

A similar requirement for bicarbonate has been reported by Klein[21] in his studies on the synthesis of fatty acids by particulate preparations from yeast cells and by Squires and co-workers[70] in their studies on fatty acid synthesis on extracts from avocado fruit. This common requirement for fatty acid synthesis establishes the universality of the fatty acid synthesizing system in living cells.

Recently, Wakil was able to isolate for the first time an intermediate in the synthesis of long-chain fatty acids from AcCoA and thus split the reaction into two parts.[32] When AcCoA was incubated with R_{1gc} in the presence of HCO_3^-, Mn^{++} and ATP, an intermediate which can be converted in the presence of R_{2gc} and TPNH to palmitate, was isolated from the reaction mixture. This intermediate incorporated both C^{14}-acetyl CoA and $HC^{14}O_3^-$ in an approximate ratio of 1:1. On hydrolysis with alkali, the saponifiable fraction was extracted with diethyl ether and was shown to contain all of the radioactivity. This acid was identified as malonate[32] by its R_f on two different chromatographic systems, by its melting point and by the melting point of the *p*-nitrobenzyl derivative.

The formation of malonyl derivative is dependent on the presence of Mn^{++}, ATP and HCO_3^- (Table V). Thus it is apparent that ATP activates the HCO_3^- (or any one of its equilibrium species, CO_2 and H_2CO_3) which is then condensed with the methyl group of AcCoA to form malonyl CoA. For this reason

TABLE IV
Failure of $H^{14}CO_3^-$ to Become Incorporated into the Synthesized Fatty Acids

No. of Experiment	Additions to Complete System	TPNH ($-A_{340\ m\mu}$)	AcCoA Incorporated (c.p.m.)	$H^{14}CO_3^-$ Incorporated (c.p.m.)
1	Acetyl-1-C^{14} CoA and unlabeled HCO_3^-	0.460	8,430	...
	Unlabeled acetyl CoA and $HC^{14}O_3^-$ (6.10^5 counts/min.)	0.420	...	50
2	Acetyl-1-C^{14} CoA and unlabeled HCO_3^-	0.375	6,800	...
	Unlabeled acetyl CoA and $HC^{14}O_3^-$ (2.10^6 counts/min.)	0.370	...	0.0

Each cuvette contained the following components of the complete system: 25 μM of potassium phosphate (pH 6.5), 1 μM ATP, 0.3 μM of $MnCl_2$, and 0.05 μM of TPNH. As indicated, the following reagents were also added: 50 mμM of acetyl-1-C^{14} CoA (37,000 counts/min.) or 45 mμM of unlabeled AcCoA, 4 μM of either unlabeled HCO_3^- or $HC^{14}O_3^-$. Total volume was 0.5 ml. The reaction was started by the addition of 0.6 mg. of R_{1g} and 0.5 mg. R_{2g}. The change in optical density at 340 mμ was followed for five minutes at 30°c. in the Beckman DUR, and the amount of fatty acid synthesis was determined as usual at the end of this time.

the name acetyl CoA carboxylase (referred to previously as R_{1gc}) has been suggested for this enzyme.

The product of the carboxylation reaction has been identified as monomalonyl CoA, by its behavior on paper chromatography in two different systems (isobutyric acid:ammonia and ethanol:sodium acetate), which is identical to chemically prepared malonyl CoA. This evidence led us to conclude that the first step in the fatty acid synthesis is the formation of malonyl CoA from bicarbonate and AcCoA. Recently, Brady[71] has reported his studies concerning the possible formation of higher fatty acids (as identified by the R_f of their hydroxamic acids) from malonyl CoA by either crude R_1 or R_2 fractions. Ganguly[72] was able to study, with the aid of malonyl CoA, the distribution of the non-mitochondrial system in various tissue. The results (Table v) indicate that there is a wide distribution of this system in animal tissues which may suggest that this system is the main pathway for synthesis of fatty acids.

Possible Mechanisms of Fatty Acid Synthesis

As indicated previously, malonyl CoA, in the presence of TPNH, can be converted to palmitate by the second enzyme fraction (R_{2gc}). This conversion can be followed either spectrophotometrically by measuring the oxidation of TPNH or isotopically by the incorporation of C^{14}-labeled malonyl CoA into palmitate (Fig. 4). The addition of AcCoA to the reaction mixture results in an increase at the rate of oxidation of TPNH with a concomitant increase in the amount of in-

TABLE V
Distribution of the Non-Mitochondrial System in Crude Extracts of Various Tissues

Tissue (crude extract)	Malonyl CoA Converted to Palmitate/mg. of Protein/10 Minutes (mμM)
Beef liver	0.15
Beef brain	1.8
Beef pancreas	0.3
Beef lung	0.23
Beef kidney	0.06
Beef small intestine	0.02
Beef mammary gland	5.3
Beef adipose tissue	2.3
Beef suprarenal fat	7.0
Beef aorta	0
Chicken liver	14.5
Chicken ovary	0.04
Chicken oviduct	0
Pigeon liver	28.9

NOTE: Each assay tube contained 20 μM of potassium phosphate buffer (pH 6.5), 50 μM of TPNH, 6 mμM (12,000 c.p.m. total activity) of labeled malonyl CoA, limiting amount of the crude extract of the tissues indicated and water to 0.40 ml. The reaction mixture was incubated at 38°c. for ten minutes.

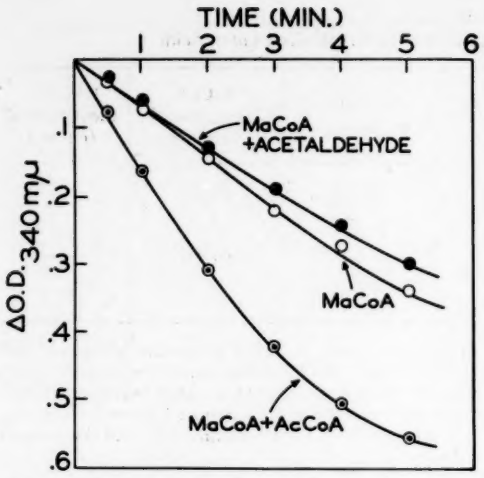

FIG. 4. Each cuvette contained 20 μM of potassium phosphate buffer (pH 6.5), 50 mμM of TPNH, 50 mμM of synthetic unlabeled malonyl CoA, 200 μg. of R_{2gc} and H_2O to 0.4 ml. Where indicated, 1,000 mμM of acetaldehyde and 100 mμM of AcCoA were added. (MaCoA = malonyl CoA.)

corporation of C^{14}-labeled malonyl CoA into palmitate (Fig. 4). Furthermore, a significant amount of C^{14}-acetyl CoA is incorporated into palmitate when unlabeled malonyl CoA is used as shown in Table VI. It is also apparent from the data in Table VI that the amount of AcCoA introduced into palmitate corresponds to about one-eighteenth of the total amount of TPNH oxidized which compares favorably with the theoretic value of 14, assuming that two TPNH molecules are required for the complete reduction of the carboxyl group of AcCoA to the methylene group.[68] In other words, one "C_2 unit" or two carbon atoms of palmitate is derived from AcCoA and the remaining fourteen carbon atoms are derived from malonyl CoA. These findings suggested the possibility that AcCoA may constitute the last carbon pairs (carbon atoms 15 and 16) of palmitate.[33]

The preparation of R_{2gc} used contains an enzyme which decarboxylates malonyl CoA to CO_2 and AcCoA (as measured by the enzymatic formation of citrate[73]).[74] This would explain why malonyl CoA, in the absence of added AcCoA, can form palmitate in the presence of R_{2gc} and TPNH (Table VI).

TABLE VI

Addition to Reaction Mixture*	TPNH Oxidized (mμM)	C^{14}-Substrate Incorporated into Palmitate (mμM)
None	18.0	..
1-C^{14}-acetyl CoA (12 mμM)	32.2	1.80
1-C^{14}-acetyl CoA (12 mμM) plus acetaldehyde (1,000 mμM)	32.2	1.90
1-C^{14}-acetate (320 mμM)	18.0	0.00
2-C^{14}-malonate (1,000 mμM)	18.2	0.00

* Each cuvette contained 20 μM of potassium phosphate buffer (pH 6.5), 50 mμM of TPNH, 50 mμM of synthetic unlabeled monomalonyl CoA, 200 μg. of R_{2gc}, H_2O to 0.04 ml. and the added substrates as indicated. The mixture was incubated for ten minutes at 38°c. in each experiment.

Acetaldehyde does not substitute for AcCoA under these conditions (Fig. 4), nor does it affect the incorporation of C^{14}-acetyl CoA into palmitate (Table VI). These results are contrary to Brady's proposed mechanism for fatty acid synthesis[71] which implicates acetaldehyde as an intermediate.

On further purification of the R_{2gc} fraction by rechromatography on cellulose column, we were able to demonstrate that the synthesis of palmitate is absolutely dependent on the simultaneous presence of AcCoA, malonyl CoA and TPNH. Acetyl CoA can be substituted by propionyl CoA but not by any of the other acyl CoA's. For instance, labeled butyryl CoA, hexanoyl CoA or octanoyl CoA cannot substitute for AcCoA. This is contrary to our earlier reports[33] that AcCoA can be substituted by butyryl CoA or octanoyl CoA; in these studies, relatively cruder preparations of R_{2gc} were employed.

The Michaelis-Menten constants for AcCoA and propionyl CoA were determined in the usual manner and were found to be 2.3×10^{-6} M, and 5.7×10^{-5} M, respectively. These values indicate that the affinity for the condensation of malonyl CoA onto propionyl is one twenty-fifth that of AcCoA, and it decreases sharply with the use of butyryl CoA to a value almost nil.

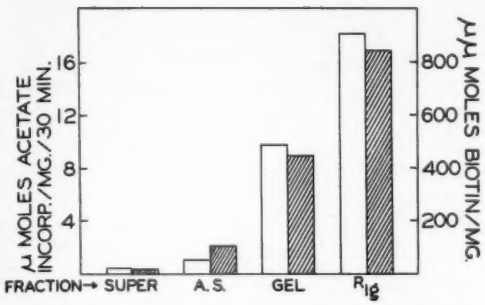

FIG. 5. The specific enzyme activity of R_1 fractions (AcCoA carboxylase, left column) is compared with the concentration of protein-bound biotin (right column). The specific activity is expressed in terms of μM of acetate incorporated into fatty acid per mg. of R_1 fraction protein per 30 minutes. Analyses of biotin were carried out on the same set of R_1 fractions.[79]

The stoichiometry for the condensation of AcCoA and malonyl CoA shows that for each mole of palmitate synthesized, there are 1 mole AcCoA, 7 moles of malonyl CoA and 14 moles of TPNH consumed. The over-all reaction can be presented as follows:

$$CH_3COSCoA + 7\ HOOCCH_2COCoA + 14\ TPNH + 14\ H^+ \rightarrow CH_3(CH_2)14COCoA + 14\ TPN^+ + 7\ CO_2 + 7\ COASH + 7\ H_2O$$

When propionyl CoA was used instead of AcCoA, the product of the synthesis is an odd-chain fatty acid with seventeen carbon atoms. Therefore, this observation explains the occurrence of the odd-chain fatty acid in animal tissues which appears to be primarily dependent on the availability of propionyl CoA in the cell rather than on the specificity or the nature of the enzyme system.

In our preliminary report[33] we proposed a hypothetical scheme for the synthesis of palmitate from AcCoA and malonyl CoA. In this scheme we proposed the formation of a C_5 intermediate (possibly acetomalonyl CoA) as the first intermediate in the condensation of AcCoA and malonyl CoA. We have not been able to isolate such an intermediate, nor have we been able to accumulate any intermediate in this scheme of reactions. Since short-chain fatty acids (i.e., butyryl CoA, hexanoyl CoA, etc.) or their substituted derivatives do not incorporate into palmitate, nor do they accumulate in this system (our attempts to trap these acyl CoA's failed), they have been excluded as intermediates in the overall synthesis. Some remaining possibilities are the following: the formation of dicarboxylic acid with each phase of condensation which is ultimately decarboxylated to palmitic acid; the formation of poly-keto C_{16} polymer which is then reduced by TPNH to palmitate; or the formation of acyl-S–enzyme which does not equilibrate with free acyl CoA (C_4, CoA, C_6CoA, C_8CoA etc.), nor would it dissociate from the enzyme until palmitate is synthesized. The poly-keto C_{16} acid was advocated by earlier organic chemists and was suggested recently by Lynen,[75] who later changed his views in favor of the acyl-S–enzyme hypothesis.[76] At the present time, the poly-keto acid hypothesis appears to be attractive to us and efforts are being made to isolate such an intermediate. There are many and serious objections, from the enzymatic point of view, to these hypotheses and the final answer must await further experimentation.

ROLE OF BIOTIN IN FATTY ACID SYNTHESIS

Available information concerning the role of biotin in metabolic reactions points to a close relationship between this vitamin and the metabolism of carbon dioxide in the carboxylation-decarboxylation type of reactions.[77] A relationship has also been reported to exist between biotin and the metabolism of fatty acids. In this relationship such unsaturated fatty acids as oleic and linoleic acids are able to promote growth of many micro-organisms in the absence of biotin.[78] Saturated fatty acids, although inactive alone, have a sparing effect on the unsaturated fatty acids. Until recently[79,80] this relationship between biotin and the metabolism of fatty acids was obscure and could not be related superficially to the more generalized effect of biotin on the metabolism of carbon dioxide. The discovery by Gibson, Titchener and Wakil,[25] that bicarbonate is an absolute requirement for the synthesis of fatty acids by the purified enzyme system, has suggested a possible role for biotin in the system. Indeed, when the enzyme fractions were assayed for biotin by the procedure of Wright and Skeggs,[81,82] there was

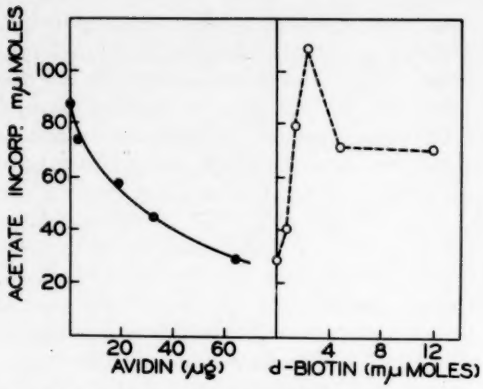

FIG. 6. Reversal of the avidin inhibition of fatty acid synthesis by *d*-biotin. The left curve indicates the addition of increasing amounts of purified avidin to a series of identical incubation mixtures. The maximum inhibitory effect in this sequence was obtained with 64 μg. avidin. The right curve illustrates that by adding increasing small quantities of free *d*-biotin to a second series of incubation mixtures (each containing 64 μg. avidin), the inhibitory effect of avidin on synthesis is progressively eliminated. Each experimental system contained (in a volume of 0.50 ml.) the following reagents: potassium phosphate buffer (pH 6.5), 40 μM; $MnCl_2$, 0.3 μM; cysteine, 6 μM; ATP, 2.7 μM; TPN, 0.38 μM; isotrate, 6 μM; CoASH, 0.04 μM; acetate-1-C^{14}, 1.32 μM; $KHCO_3$, 10 μM; acetic thiokinase (contains isocitric dehydrogenase), 0.3 mg.; and R_{2s}, 0.09 mg. The reactions were initiated by the addition of 0.24 mg. R_{1g}. Incubations were carried out at 38°c. for thirty minutes in an atmosphere of nitrogen.

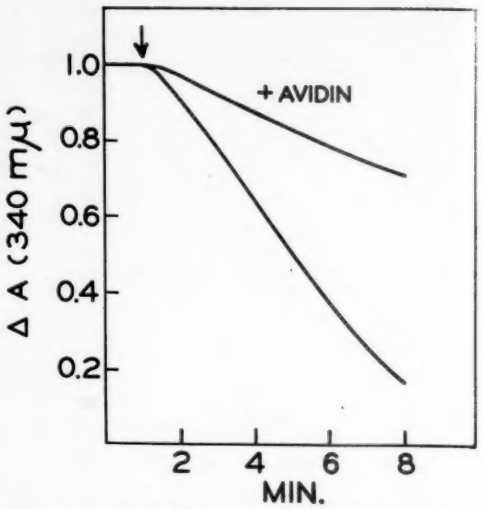

FIG. 7. Avidin inhibition of the oxidation of TPNH associated with fatty acid synthesis. Purified avidin (46 μg.) was added to one of two identical systems. Each system contained (in a total volume of 0.50 ml.) the following reagents: potassium phosphate buffer (pH 6.5), 50 μM; $MnCl_2$, 0.3 μM; ATP, 1 μM; AcCoA, 0.21 μM; $KHCO_3$, 5 μM; TPNH, 0.17 μM; and R_{2g}, 1.5 mg. The reaction was initiated in both systems by the addition of 0.26 mg. R_{1g} (indicated by arrow). The optical density at 340 mμ (1.0 cm. cell) was followed continuously in the Beckman DUR spectrophotometer (at 38°c.).

a significant concentration of this vitamin in one of the fractions, namely, R_{1gc} (malonyl carboxylase).[25,79,80] Biotin concentrates with the active protein of this fraction in each phase of purification (Fig. 5), and the ratio of enzymatic activity to the content of biotin remains essentially the same throughout the process of purification. The final concentration of biotin in the most purified preparation (after purification by ion exchange column) amounts to about 2 moles or 3 moles of biotin per 10^6 gm. of protein or 1 mole of biotin per 300,000 to 500,000 gm. of protein, which is the highest protein-bound biotin reported.

Biotin is tightly bound to the protein and can be released only by tryptic digestion or acid hydrolysis.[80] The product of tryptic digestion is not free biotin but a conjugated derivative or biotin,[80] as determined by microbiologic assay[81] and paper chromatography.[82] Free biotin is released by the acid hydrolysis of the protein fractions or the tryptic digest of the enzyme.[80]

Our recent studies[79,80] verify that the protein-bound biotin does indeed participate in the over-all synthesis of long-chain fatty acids from AcCoA. We have demonstrated that the conversion of AcCoA to palmitate is inhibited by avidin,[77] an egg-white protein which specifically binds biotin tightly (Fig. 6). This inhibition can be relieved by the addition of free *d*-biotin (Fig. 6). The effect of avidin can be also demonstrated spectrophotometrically (Fig. 7).

Animals deficient in biotin, rats or chicks, were prepared[83] and their livers were used to prepare the fatty acid synthesizing system. The results indicate that the levels of acetyl CoA carboxylase in the livers of these animals are lower than the amount of carboxylase in

the normal animals. On purification of the enzyme from deficient animals, the specific activity of the enzyme increases as well as the concentration of biotin. At the highest level of purity, the content of biotin of such enzyme preparations appears to be comparable to that of the preparations from normal animals. This observation indicates that biotin deficiency, however severe it may be, results in a decrease of the total level of the acyl CoA carboxylase, but it does not completely eliminate this vital enzyme from the liver.

Lynen and his collaborators[84] recently have isolated another biotin-containing enzyme which carboxylates β,β-dimethyl acrylyl CoA to form β-methyl glutaryl CoA. They proved that biotin is an integral part of this enzyme and that CO_2-biotin–enzyme is an intermediate in this reaction. The proposed scheme is as follows:

$$CO_2 + ATP + \text{biotin-enzyme} \rightarrow CO_2 \sim \text{biotin-enzyme} + P_i + ADP$$

$$CO_2 \sim \text{biotin-enzyme} + \beta,\beta\text{-dimethyl acrylyl CoA} \rightarrow \beta\text{-methylglutaryl CoA} + \text{biotin-enzyme}$$

A similar mechanism may be operative in the carboxylation of AcCoA to form malonyl CoA.

MAMMARY GLAND SYSTEM

Popják and Tietz[52,53,85] have studied fatty acid synthesis in homogenates and later in soluble preparations from the mammary gland of lactating rats. The soluble supernatant fraction exhibits greater activity in synthesizing fatty acids from acetate than does the full homogenate. Neither the mitochondrial nor the microsomal fractions were required for fatty acid synthesis. ATP was absolutely required for synthesis; and when the enzyme preparation was treated with Dowex-1–Cl resin, a requirement for CoA and DPN was noted. Oxalacetate, α-ketoglutarate and succinate markedly stimulated the synthesis. Malonate noticeably stimulated the synthesis of fatty acids and, in combination with α-ketoglutarate, it increased the amount of C^{14}-acetate incorporated into the fatty acids thirty fold. The extract of the mammary gland synthesizes both short- (C_4 to C_8) and long-chain fatty acids (C_{12} to C_{18}) while the whole homogenate preparation synthesizes predominantly long-chain fatty acids.[49]

Hele, Popják and Lauryssens[86] prepared, from the mammary glands of lactating rabbits, a fatty acid synthesizing system which had essentially the same characteristics as did the previous systems of rats and sheep. On fractionation with ammonium sulfate (35 to 65 per cent) an enzyme preparation was obtained which synthesized short-chain fatty acids from acetate in the presence of ATP, CoA, Mg^{++}, cysteine and DPNH. These investigators were unable to demonstrate any requirement for TPN^+ or for TPNH. Furthermore they have shown that predominantly short-chain fatty acids are synthesized by the ammonium sulfate fractions of the mammary gland[86,87] and that the relative amounts of octanoate and hexanoate synthesized are much smaller than the amount of β-hydroxyoctanoate.[87] In other words, the amounts of saturated fatty acids formed, except for butyrate, are extremely small in comparison with the amounts of β-hydroxy and unsaturated acids. It is of great interest to note in the description by Popják and Tietz[52,53,84] that in the crude homogenates the fatty acid synthesizing system of the mammary gland is remarkably similar to that described earlier of the livers of the pigeon, rat and chicken. It may be possible that the mammary gland has two soluble synthesizing systems of fatty acids; one system for the synthesis of the short-chain acids (described by Hele and Popják[86]) and the other is the non-mitochondrial system which is similar to the system described by Wakil et al.[49] Further information is needed in order to ascertain this point.

SUMMARY

Evidence has been presented to show that there are two distinct systems for the synthesis of fatty acids. (1) The mitochondrial system is located in the mitochondria and involves the enzymes of the β-oxidation system (thiolase, enoyl hydrase, β-hydroxyacyl dehydrogenase) working in reverse plus the TPN-α,β-unsaturated fatty acyl CoA reductase enzyme. Both TPNH and DPNH are re-

quired for the synthesis. Essentially this system is for the elongation of the existing fatty acids by the addition of two-carbon units at a time. It is possible that this system may be responsible for the formation of stearate from palmitate, arachidonate from linoleate, etc.

(2) The non-mitochondrial system is located in the cytoplasm of the cell and catalyzes the conversion of AcCoA to palmitate in the presence of ATP, Mn^{++}, HCO_3^- and TPNH (DPNH may be substituted for TPNH at a slower rate). Acetyl CoA is condensed with HCO_3^- to form malonyl CoA, a reaction which is catalyzed by acetyl CoA carboxylase (a biotin-containing enzyme) in the presence of ATP and Mn^{++}. The biotin is bound to the protein and evidence has verified that it does participate in the formation of malonyl CoA. Malonyl CoA condenses with acetyl CoA to form several intermediates which can be reduced by TPNH to form the saturated fatty acids.

This system appears to be the main pathway for fatty acid synthesis, and it is widely distributed in living organisms. So far this system has been isolated from pigeon liver, chicken liver, rat liver, rat kidney, yeast cells and avocados.

The enzymic system for the synthesis of short-chain fatty acids in the mammary gland may be different from the aforementioned systems, but more information is needed before final judgment can be made.

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DISCUSSION

DR. DONALD S. FARNER (*Pullman, Washington*): I noticed in this list of tissues, from which you said you could get the enzyme system, you did not mention any skeletal muscle. Have you tried this system with respect to pectoral muscle in pigeon?

DR. WAKIL: No. We have not done any work on skeletal muscle with this system.

DR. JAY TEPPERMAN (*Syracuse, New York*): It has been suggested that the "old-fashioned" or "two-years-ago" type of fatty acid synthesis might go on in the mitochondria whereas the system that you discussed is located in the extramitochondrial portion of the cell. Dr. Wakil, do you believe any significant amount of fatty acid synthesis takes place in the mitochondrial environment, which is notoriously low in the reduced forms of the necessary coenzymes for reductive synthesis?

DR. WAKIL: We believe—and we have some data to support this notion—that mitochondria may carry on fatty acid synthesis by the reversal of β-oxidation. However, I think mitochondria are primarily concerned with the elongation of fatty acid by the addition of C_2 units to the pre-existing acyl coenzyme A (CoA).

Palmitate is the product of the non-mitochondria system; while stearate, oleate, arachidonate, and so on, are the product of the mitochondrial system.

UNIDENTIFIED SPEAKER: The main part is soluble?

DR. WAKIL: This system is a soluble system.

DR. MARVIN D. SIPERSTEIN (*Dallas, Texas*): In

view of the demonstration by Dr. Wakil that fatty acid synthesis could go on in the supernatant soluble fraction of liver by a mechanism different from that in mitochondria, we have attempted to see whether the mitochondria system was of any physiologic significance.

First of all, I think it should be pointed out that the levels of TPNH in the mitochondria are at least as high as those in the supernatant. This still can be considered as a reductive atmosphere from the standpoint of the TPNH.

We have found that approximately an equal amount of acetyl CoA derived from pyruvate 2-C^{14} can be converted to fatty acids in the mitochondria and in the supernatant.

In answer to the logical objection that this might be due to elongation of palmitate to stearate, isolation of these counts by gas chromatography has shown that the counts are indeed in palmitic acid.

DR. WAKIL: We have found that we can make palmitate as well as other things. However, in our hands, the requirement of acyl CoA, such as butyryl CoA or hexanoyl CoA, was almost obligatory for the incorporation of acetyl CoA by the mitochondria into the higher chain acids. Without this ATP had to be added. This we interpreted as being due to the residual fatty acids.

Have you found the distribution of C^{14} palmitate to show that it is a *de novo* synthesis, that is, all the molecule derived its radioactivity from the acetate?

DR. SIPERSTEIN: No, we have not done a carbon degradation, and the objection of Dr. Wakil from this standpoint is quite valid. However, the major mechanism of elongation probably would involve palmitic acid to stearic acid. As such, this is one bit of evidence that this is *de novo* synthesis though not definitive.

DR. RACHMIEL LEVINE (*Chicago, Illinois*): Dr. Wakil, there has been some old evidence that at least in mammary gland one can isolate even-numbered intermediates from four up. Is this provided for in your scheme, and is this true?

DR. WAKIL: No, this is not provided for by the scheme presented here for the soluble enzyme system. But the short-chain fatty acids (C_4, C_6, etc.) can be synthesized by the mitochondrial system.

DR. RABEN (*Needham, Massachusetts*): I wonder whether the system of Dr. Wakil is going to make it harder to account for the large amounts of aceto-acetic acid that appear, say, in diabetes and other conditions. I think we have all been led to believe that they are now largely recondensation products of acetate.

DR. WAKIL: It is true that it is a recondensation product of acetate. However, the system for the formation of acetoacetate or betahydroxybutyrate, the so-called ketone bodies, has been well studied by Lynen in Germany and Minor Coon in this country; it involves the intermediacy of a C_6 dicarboxylic acid which is then cleaved to acetoacetate.

DR. LEVINE: In other words, this is a side branch of the first portion of that scheme?

DR. WAKIL: It has nothing to do with this scheme.

DR. LEVINE: Nothing whatsoever, even in the original condensation?

DR. WAKIL: In the whole condensation formed, from our studies, this is not part of this mechanism, although in the animal everything is well interrelated. Schematically on the enzymatic or molecular level, it seems to have nothing to do with this system.

The Homeostatic Control of Cholesterol Synthesis in Liver

MARVIN D. SIPERSTEIN, M.D., PH.D.*

DESPITE wide fluctuations of cholesterol in their diets, most animals are able to maintain tissue and plasma concentrations of cholesterol at fairly constant levels. It was shown almost ten years ago by Gould[1] and subsequently confirmed by both Tomkins et al.[2] and Frantz et al.[3] that this constancy of cholesterol concentration could be explained, at least in part, by the fact that an increase in ingested cholesterol results in a marked decrease in the rate at which cholesterol is synthesized by the liver.

These earlier observations could be readily confirmed in that we have been able to demonstrate repeatedly that feeding rats a 2.5 or a 5 per cent cholesterol diet for from twelve hours to as long as one month will result in a ten to 300 fold depression in cholesterol synthesis by the livers of such animals. As indicated in Figure 1, these observations indicate that the body must normally possess a sensitive feedback mechanism by which exogenous cholesterol, and presumably endogenous cholesterol, serve to block one or more of the reactions involved in the conversion of acetate to cholesterol. As a result, cholesterol synthesis is decreased and a homeostatic control of the cholesterol concentration of the body is thereby maintained.

The mechanism of this negative feedback reaction is not known. As an initial approach to a study of this process, attempts have been made to localize the site or sites on the pathway of cholesterol synthesis at which this physiologic regulation of cholesterogenesis occurs.[4–6] Approximately twenty-six separate reactions are believed to be involved in the biochemical conversion of acetate to cholesterol. These can be briefly summarized as follows:

$$\text{acetate} \xrightarrow{4\ \text{steps}} \text{mevalonic acid} \xrightarrow{7\ \text{steps}} \text{squalene} \xrightarrow{15\ \text{steps}} \text{cholesterol.}$$

The approximate location of the cholesterol-induced block of cholesterol synthesis was examined by determining the effect of feeding a 2.5 or 5 per cent cholesterol diet on the ability of rat liver slices to carry out these major steps of cholesterol synthesis. Carbon^{14}-labeled acetate, mevalonate or squalene were the labeled substrates employed to measure the rates of reaction. The results of a typical experiment are illustrated in Figure 2. As shown in experiment 1, the feeding of a 2.5 per cent cholesterol diet for eleven days depressed the over-all conversion of acetate to cholesterol by a factor of thirty-four (Fig. 2). The influence of cholesterol feeding on the reactions involved in the conversion of squalene-C^{14} to cholesterol is shown in experiment 2, Figure 2. This phase of cholesterol synthesis was found to be either unaffected or, as in this example, slightly increased by the feeding of cholesterol. The major site of the cholesterol block is not, therefore, in one of the fifteen reactions involved in the conversion of squalene to cholesterol. Of the eleven reactions required for the incorporation of acetate into squalene, seven can be examined by following the effect of cholesterol feeding on the conversion of mevalonate-C^{14} to squalene. As shown in experiment 3, Figure 2, dietary

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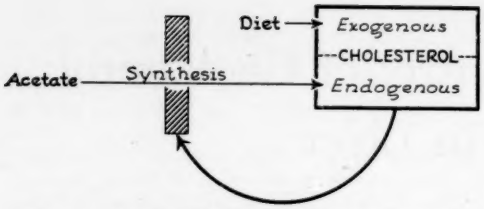

FIG. 1. Cholesterol inhibition of cholesterogenesis.

cholesterol does not inhibit these reactions, and in fact, has consistently produced a slight increase in the incorporation of mevalonate into squalene. This series of experiments suggest that the major site of the cholesterol block cannot be located in the reactions involved in the conversion of mevalonate to cholesterol.

This conclusion is further supported by the finding shown in experiment 4, Figure 2. Cholesterol feeding caused less than a 50 per cent depression in the conversion of mevalonate to cholesterol, a decrease which is not sufficient to account for the thirty-four fold depression which is seen in the over-all conversion of acetate to cholesterol. Both Gould and Popják[7] and Bucher et al.,[8,9] using similar approaches in liver homogenates, have also demonstrated that the reactions between mevalonate and cholesterol are not greatly influenced by cholesterol feeding.

It is apparent, therefore, that the site of the feedback block must be located in one of the four reactions of cholesterol synthesis located prior to the production of mevalonate.

These four reactions are believed to occur as follows:

$$\text{acetate} \xrightarrow{1} \text{acetyl CoA} \xrightarrow{2} \text{acetoacetyl CoA} \xrightarrow{3} \beta\text{-hydroxy-}\beta\text{-methylglutaryl CoA} \xrightarrow{4} \text{mevalonic acid.}$$

The site of the cholesterol block could be localized to one of these four reactions by utilizing the fact that acetoacetyl CoA serves as an important intermediate for the synthesis of long-chain fatty acids as well as for the synthesis of cholesterol, while β-hydroxy-β-methylglutaryl CoA, as has been shown by Lynen et al.,[10] is a precursor of the ketone bodies, acetoacetic acid and β-hydroxybutyric acid. A block at the site of reactions 1 or 2 should therefore lead to a depression in the conversion of acetate-C^{14} to fatty acids as

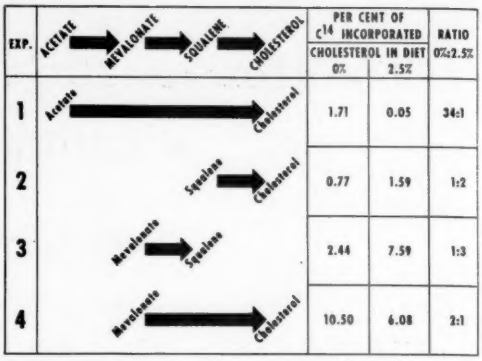

FIG. 2. Initial localization of cholesterol block.

well as to cholesterol. Similarly, a cholesterol-induced block at the site of reaction 3 would be expected to produce an inhibition in the conversion of acetate-C^{14} to ketone bodies. Studies on the effect of dietary cholesterol on these reactions were carried out, and it was found that the conversion of acetate-C^{14} to long-chain fatty acids and to ketone bodies is not inhibited significantly by cholesterol feeding. It follows, therefore, that of the four reactions involved in the conversion of acetate to mevalonate, reactions 1, 2 and 3 cannot be the site of the block in cholesterol synthesis.

As summarized in Figure 3, these findings have led to the conclusion that reaction 4 involving the conversion of β-hydroxy-β-methylglutaryl CoA to mevalonic acid is the major site at which cholesterol feeding inhibits the synthesis of cholesterol.[4,5]

The location of the cholesterol feedback mechanism at a site just beyond the last branch of the synthetic pathway would appear to be ideally suited to regulate this over-all sequence of reactions. Feedback control to a reaction beyond mevalonic acid (Fig. 3) would result in the unnecessary synthesis and perhaps the accumulation of useless intermediates whenever cholesterol was present in the diet. On the other hand, a feedback block located prior to β-hydroxy-β-methylglutaryl CoA might lead to the incidental depression of fatty acid or ketone body synthesis whenever cholesterol was fed.

Feedback mechanism, involving product inhibition of a specific early reaction site, has

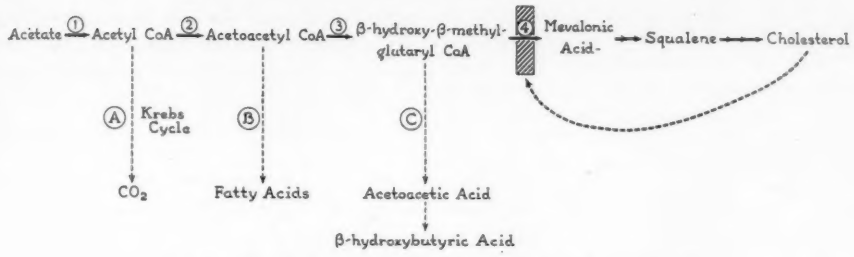

FIG. 3. Proposed site of feedback control of cholesterogenesis.

been shown to regulate synthetic processes in numerous bacterial systems[11–13]; however, as far as we are aware, such a mechanism has not been demonstrated previously in animal tissues. The finding that cholesterol synthesis is controlled by a mechanism similar to that found in bacteria suggests that this means of controlling biologic syntheses may be more widespread than has hitherto been assumed. It should be emphasized that there is already evidence to suggest that in higher animals the synthesis of fatty acids,[14] pyrimidines[15] and purine[16] may be controlled by a type of negative feedback reaction, though the location of the site or sites of control in these cases have not been determined as yet.

The exact mechanism by which cholesterol feeding inhibits the conversion of β-hydroxy-β-methylglutaryl CoA to mevalonic acid is not known; however, cholesterol itself is probably not directly responsible for the inhibition of this reaction. Feeding amounts of cholesterol sufficient to depress hepatic cholesterogenesis need not cause any elevation in total liver cholesterol.[6] Furthermore, as shown in Table I, the addition of cholesterol to normal liver slices has only a relatively small inhibitory effect on cholesterol synthesis from acetate-C^{14}. Several fatty acid esters of cholesterol have been prepared and although the addition of large amounts of these compounds caused some inhibition of cholesterol synthesis, fatty acid synthesis was likewise inhibited. This depression of cholesterogenesis appears, therefore, to be non-specific and never approaches

TABLE I

Influence on Cholesterol Synthesis of Cholesterol and Cholesterol Esters Added *in Vitro* to Normal Rat Liver Slices

Addition	Per cent Added C^{14} Incorporated Into	
	Cholesterol	Fatty Acids
Albumin, 1%	1.3	1.11
Cholesterol, 10 mg.	0.58	0.69
Cholesteryl palmitate, 10 mg.	1.23	0.86
Cholesteryl linoleate, 10 mg.	0.26	0.25
Cholesteryl arachidonate, 10 mg.	0.79	0.28

NOTE: Each flask contained one of the above cholesterol derivatives dissolved in 1% albumin plus 5 ml. Krebs-Bicarbonate buffer, 5 ml., 500 mg. liver slices and 1 μM acetate-2-C^{14} (0.5 μC). The flasks were incubated two hours at 37°C.

TABLE II

Influence on Cholesterol Synthesis of Bile Acids Added *in Vitro* to Normal Rat Liver Slices

Experiment	Addition	Per cent Acetate-1-C^{14} Converted to		
		Cholesterol	Fatty Acids	CO_2
1	Nothing	0.23	1.51	32.5
2	Taurocholic acid 10^{-5}M	0.26	1.88	36.2
3	Taurocholic acid 10^{-4}M	0.30	1.56	35.1
4	Taurocholic acid 10^{-3}M	0.32	2.46	37.2
5	Taurocholic acid 10^{-2}M	0.25	1.39	35.6
6	Cholic acid 10^{-4}M	0.28	1.30	35.0
7	Cholic acid 10^{-3}M	0.05	0.10	11.3

NOTE: Each flask contained the concentration of bile acid noted above plus 500 mg. liver slices and 10 μM acetate-1-C^{14} (1 μC) in 5 ml. Krebs-Bicarbonate buffer. The flasks were incubated for two hours at 37°C.

TABLE III
Effect of Cholesterol Synthesis of Feeding Taurocholic Acid for Twenty-three Hours

Experiment	Taurocholic Acid in Diet	Per cent Acetate-1-C^{14} Converted to		
		Cholesterol	Fatty Acids	CO_2
1	0	0.59	2.46	43
2	0	1.33	3.41	39
3	0	0.74	1.08	40
4	0	0.38	1.66	39
5	2.5%	0.63	0.83	35
6	2.5%	0.32	0.39	39
7	2.5%	0.35	0.74	37
8	2.5%	0.22	1.29	34

NOTE: Each flask contained 500 mg. of liver slices in 5 ml. Krebs-Bicarbonate Buffer plus 10 μM acetate-1-C^{14}. The flasks were incubated for two hours at 37°c.

the marked inhibition produced by cholesterol feeding.

Since the major biochemical route by which the cholesterol is metabolized in the rat is via taurocholic acid,[17,18] attempts have been made to determine whether this breakdown product of cholesterol might be the direct mediator of the inhibition of cholesterol synthesis. As is shown in Table II, taurocholic acid, when added to liver slices even at a concentration of 10^{-2}M, had no effect on cholesterol synthesis. Cholic acid at a level of 10^{-3}M depressed cholesterol synthesis somewhat, but this effect is not specific since a definite depression in lipogenesis was produced also at this bile acid concentration. Similarly, the feeding of taurocholic acid at a concentration of 2.5 per cent for a period of twenty-three hours produced only a small depression of cholesterogenesis (Table III). Administration of cholesterol at this level for only twelve hours will cause at least a tenfold depression in cholesterol synthesis.* The exact mechanism by which cholesterol feeding blocks the conversion of β-hydroxy-β–methylglutaryl CoA to mevalonic acid and thereby inhibits cholesterogenesis is still to be worked out; further studies of this problem are currently in progress.

* Behers et al.[19–21] have found that feeding rats or mice an 0.5 per cent cholic acid diet for from twelve hours to three weeks causes some depression in cholesterol synthesis; however, this effect is not great, and, as suggested by these authors,[20] is probably secondary to the elevation of hepatic cholesterol which results from feeding such diets.

SUMMARY

The feedback mechanism by which cholesterol synthesis is regulated in liver has been studied. Evidence has been presented which indicates that exogenous cholesterol inhibits cholesterol synthesis primarily by blocking the conversion of β-hydroxy-β–methylglutaryl CoA to mevalonic acid. This reaction would appear to represent the major biochemical site of normal homeostatic control of cholesterol synthesis in the liver. This inhibition of cholesterol synthesis does not appear to be directly mediated by cholesterol itself, the common cholesterol esters, or taurocholic acid. The exact mechanism by which cholesterol feeding inhibits the conversion of β-hydroxy-β–methylglutaryl CoA to mevalonate remains to be elucidated.

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DISCUSSION

DR. O'CONNELL (*The Upjohn Company*): I believe this step is the same one that Nancy Bücher found to be inhibited in the starvation of animals. They seem to lose their ability to synthesize cholesterol. If this is so, this seems to be a particularly sensitive step in two directions, both in feeding and in starvation.

Do we know enough yet about the kinetics of the overall process to know if this is a rate-determining step in the whole process?

DR. SIPERSTEIN: The only additional evidence that I can cite to support the generalization that this is the important step in the control of cholesterol in a variety of circumstances, is by Fletcher and Myant who showed that thyroxin may act at a step between acetate and mevalonic acid, and I proffer a guess that the HMG-CoA reductase will be the actual site, when localized, for the action of thyroxin on cholesterol synthesis.

The action of glucose in controlling cholesterol synthesis may also be at this site. Glucose has been shown to affect cholesterol synthesis by the production of TPNH. And, as you will note, reduced triphosphopyridine nucleotide (TPNH) is involved in β-hydroxy-β–methylglutaryl CoA reduction to mevalonic acid.

I should state categorically, that however, the cholesterol feedback mechanism, which I just described, does not involve TPNH since added TPNH will not reverse the feedback in broken-cell preparations. The glucose mechanism of control, however, does involve TPNH, rather than a direct enzymatic effect.

DR. JAMES SALTER (*Toronto, Canada*): Is it the cholesterol *per se* that actually suppresses its synthesis, or some product of cholesterol catabolism, if there is such a thing, or something involved in the excretion of cholesterol by the bile salts?

It is not often that a product inhibits by itself. With urea synthesis, inhibition is brought about by the automatic formation of pyruvic acid under some circumstances.

DR. SIPERSTEIN: In bacterial systems, it is usually the product which feeds back to inhibit the reaction. It can inhibit in one of several ways. The product could inhibit the enzyme reaction directly by blocking the enzyme, competitively or noncompetitively. Such a mechanism has been shown to operate in several bacterial systems, including the one described by Yates and Pardee for pyrimidine synthesis. It is probably true for adenine synthesis, as well.

Likewise, the feedback mechanism might operate by blocking the synthesis of the enzyme, and this mechanism has been shown in bacterial systems also.

The addition of cholesterol *in vitro* has had no effect on this reaction, so that we do not feel that this is a direct effect of cholesterol on the enzyme, the β-hydroxy-β–methylglutaryl CoA reductase.

We have synthesized cholesterol palmitate and cholesterol oleate, linoleate and arachidonate, and none of these, added *in vitro*, have an inhibiting effect on the synthesis of cholesterol.

The addition of the common bile acids or their amide complexes will not inhibit the reaction in physiologic amounts.

Finally, I would speculate that since it takes at least twelve to sixteen hours of cholesterol feeding to produce the inhibition, it would seem likely that inhibition of enzyme synthesis might be involved.

DR. RACHMIEL LEVINE (*Chicago, Illinois*): Dr. Siperstein, your objection to the idea that cholesterol would act by mass action was your demonstration that the cholesterol level does not increase. How does the enzyme know that you are feeding cholesterol?

DR. SIPERSTEIN: The other evidence that cholesterol does not act by mass action is, of course, the demonstration that the last sequence of reactions is not involved.

In order to explain such a process, which apparently comes up in designing electric feedback mechanisms, an amplifier is needed to detect minute amounts of air in the feedback mechanism.

One could picture such an amplifying system operating by using the concept which Monod has advocated for enzyme induction or Vogel has advocated for enzyme repression. Small amounts of cholesterol could get into the RNA template which is responsible for the synthesis of β-hydroxy-β–methylglutaryl CoA reductase and plug the template so that many molecules of enzyme cannot then be manufactured.

DR. LILLIAN RECANT (*St. Louis, Missouri*): If you took an animal that had nephrosis or hypothyroidism, in which you have elevated levels of cholesterol, would this feeding mechanism inhibit the reaction?

DR. SIPERSTEIN: We have not done that.

The first experiment along these lines which we have wanted to do is in human beings; namely, to study the intactness of the feedback mechanism in familial hypercholesteremic states by simply doing liver biopsies and seeing whether this reaction is inhibited.

We have now been studying the isolated reaction. We want to see whether an inhibitor is present in normal liver which is not present in familial hypercholesteremia. It is quite reasonable that a breakdown in either the sensitivity of the enzyme to the feedback mechanism or in the feedback mechanism itself might be responsible for these conditions of hypercholesteremia.

Dr. F. D. W. LUKENS (*Philadelphia, Pennsylvania*): Do you think that a difference in the effectiveness of this system accounts for the fact that hypercholesteremia can be produced in the rabbit by feeding but not in the normal dog?

DR. SIPERSTEIN: It is difficult to quantitate the sensitivity of the reaction so far as we have been carrying it out. The rabbit does possess this mechanism for feedback inhibition, but by the time we can detect it, the rabbit has already built up fairly significant amounts of cholesterol in his liver.

The reaction is probably less sensitive, but I cannot state so categorically until we really have an inhibitor and can show that *in vitro* this enzyme is more or less inhibited.

DR. RECANT: You mentioned at the beginning of your presentation that the liver was the only organ that showed this effect. Did you actually study adrenal tissue?

DR. SIPERSTEIN: We have not studied adrenal tissue.

DR. RECANT: I am interested in the possibility that in a situation such as myxedema, in which we have a diminished formation of steroid and secretion, we may be getting into some of these feedback mechanisms there, and perhaps not in the liver.

DR. SIPERSTEIN: The tissues which we have studied are: skin, which is not inhibited; and intestine, which, under the influence of cholesterol feeding, will synthesize more cholesterol than the liver because of the depression of the liver and not of the intestine.

We have not studied the endocrine organs. We have studied various sites in the intestinal tract and, for no good reason, the spleen.

Lipogenesis in Adipose Tissue

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AND WILLIAM C. STADIE, M.D.§

SINCE Wertheimer and Shapiro[1] first demonstrated that adipose tissue could synthesize fatty acids, the studies of Hausberger,[2] Feller[3] and Favarger[4] have led many to conclude that this tissue is the major site of the conversion of carbohydrate to fat. Stetten and Boxer[5] have shown that as much as 30 per cent of the daily intake of carbohydrate by the rat is converted to fat, and recent work in man and animals has assigned increasing importance to fatty acids as a substrate for the production of energy.[6] Therefore, an understanding of the regulation of fatty acid synthesis in adipose tissue would seem essential to any consideration of the overall energy balance of the intact animal.

These studies are the result of a systematic reinvestigation of the metabolism of adipose tissue *in vitro* with particular emphasis on the relationship between carbohydrate metabolism and the synthesis of fatty acid. They were stimulated by the observation that the paired epididymal fat pads of the rat permit the demonstration of an effect of insulin *in vitro* of much greater magnitude and constancy than those previously described, if the tissues are not exposed to chilled buffers and undue handling before incubation.[7]

Our initial experiments[7] demonstrated that insulin *in vitro* markedly increases glucose uptake, glucose oxidation to CO_2, and the incorporation of glucose carbon into total petroleum ether extractable lipids and long chain fatty acids. As shown in Figure 1, this effect can be demonstrated during periods of incubation as short as fifteen to thirty minutes and as long as six hours. Ball, Martin and Cooper[8] have recently confirmed the rapidity with which adipose tissue incubated under these conditions responds to the addition of insulin *in vitro*.

Insulin *in vitro* restores glucose oxidation to CO_2, and the synthesis of fatty acid from glucose in the adipose tissue of rats with alloxan diabetes.[7] It has been firmly established that either the induction of alloxan diabetes or prolonged starvation virtually abolishes fatty acid synthesis in the adipose tissue of the rat.[2,9] The addition of insulin *in vitro* to slices of liver from rats with alloxan diabetes does not correct the defects in glucose utilization and fatty acid synthesis in this tissue.[10] These observations underline the advantage of using adipose tissue as an organ in which to study the effects of insulin on the conversion of carbohydrate to fat.

The rate at which insulin, injected into rats with alloxan diabetes, restores fatty acid synthesis in adipose tissue, removed and incubated with labeled substrate *in vitro*, has recently been studied. As shown in Figure 2, both glucose oxidation to CO_2 and fatty acid synthesis from glucose are restored within two to three hours. Thus the defect in fatty acid synthesis in adipose tissue from rats with alloxan diabetes is rapidly corrected by the administration of insulin both *in vitro* and *in vivo*, and this restoration of lipogenesis is associated with the restoration of glucose utilization. These data differ from those reported by Hausberger who found that longer periods were required to restore fatty acid synthesis in the adipose tissue of rats with alloxan diabetes.[2] It should be noted that, as previously reported by Renold,[10] fatty acid synthesis in slices of liver from rats with alloxan diabetes is not

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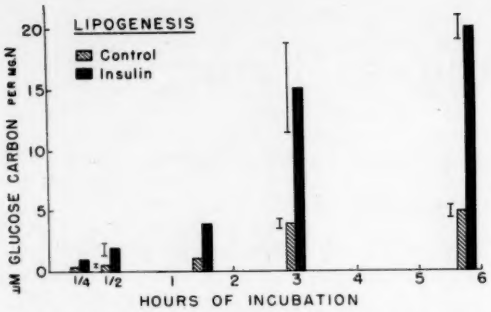

FIG. 1. Effects of insulin added *in vitro* on the incorporation of glucose carbon into petroleum ether extractable lipid, which in this instance has been shown to represent primarily long chain fatty acid,[7] after incubation periods varying from fifteen minutes to six hours. Tissues paired. Incubation carried out in Krebs bicarbonate buffer pH 7.4 containing uniformly labeled glucose-C^{14} (20 μM per ml.).

TABLE I

Effect of Insulin *in Vitro* on Glucose Metabolism of Adipose Tissue from Rats Starved Forty-eight Hours*

Glucose Carbon Isolated in:	Control	Plus Insulin	Mean Δ ± S. E. M.†
Carbon dioxide	0.61	4.03	+ 3.42 ± 0.76
Fatty acid	0.03	1.21	+ 1.18 ± 0.34

NOTE: Incubation of adipose tissue in Krebs bicarbonate buffer pH 7.4 containing uniformly labeled glucose-C^{14} in a concentration of 20 μM per ml. Tissues were paired to provide a control from the same animal for each experiment. Concentration of insulin, when present, was 0.1 unit per ml.[11]

* Data given as μM glucose carbon per mg. of N per three hours.

† Standard error of the mean.

restored to normal until six to twenty-four hours after the injection of insulin, suggesting that insulin may not have a direct effect on hepatic lipogenesis.

As shown in Table I, insulin *in vitro* stimulates glucose oxidation to CO_2 by adipose tissue from starved rats and restores the synthesis of fatty acid from glucose in this tissue.[11]

These studies underline the dependence of fatty acid synthesis in adipose tissue upon unimpaired carbohydrate metabolism.

Subsequent studies[12,13] have shown that hormones other than insulin, when added *in*

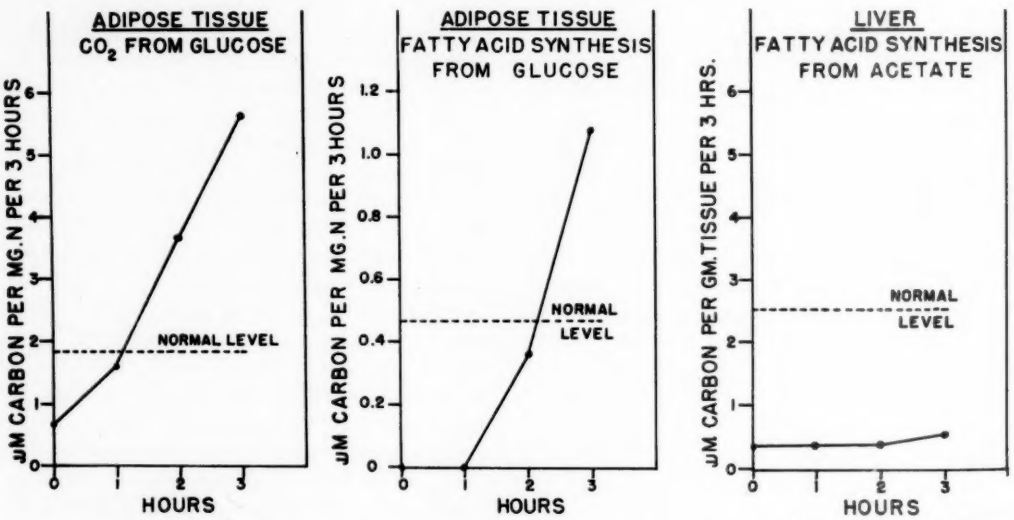

FIG. 2. Alloxan diabetic rats killed by decapitation zero, one, two and three hours after the administration of insulin *in vivo* (5 units intravenously and 10 subcutaneously). Epididymal fat pads removed and incubated in Krebs bicarbonate buffer containing uniformly labeled glucose-C^{14} (20 μM per ml.). Liver slices from the same animals were incubated in Krebs bicarbonate buffer containing acetate-1-C^{14} (4 μM per ml.). Dashed lines represent mean values for a series of normal fed controls.

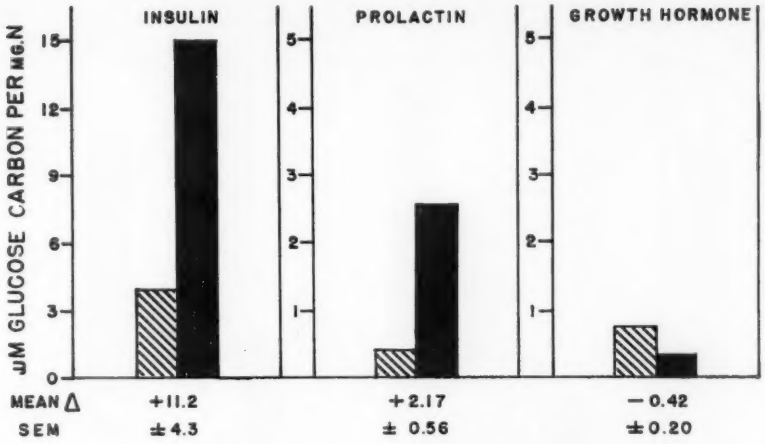

FIG. 3. *In vitro* hormonal effects on fatty acid synthesis from glucose-U–C^{14} in rat adipose tissue. Incubation carried out in Krebs bicarbonate buffer pH 7.4 containing uniformly labeled glucose-C^{14} (20 μM per ml.). Three hour incubation. Tissues paired. Striped bars indicate values for controls. Solid bars indicate values for adipose tissue to which the hormone has been added *in vitro*. Insulin, when present, 0.1 unit per ml. Ovine prolactin, when present, 1000 gamma per ml. Bovine growth hormone, when present, 1000 gamma per ml. Values expressed as μM of glucose carbon incorporated into long chain fatty acid per mg. of adipose tissue nitrogen. In the insulin experiments the values represent incorporation into total petroleum ether extractable lipids, which in this instance have been shown to represent primarily long chain fatty acid.[7]

vitro, increase the uptake of glucose and its oxidation to CO_2 by adipose tissue from fed normal rats or rats with alloxan diabetes. Ovine prolactin and bovine growth hormone in rather high concentrations consistently increase the oxidation of glucose carbon to CO_2 while bovine serum albumin in the same concentration has no consistent effect.[12] As shown in Figure 3, prolactin *in vitro* stimulated the incorporation of glucose carbon into long chain fatty acid by adipose tissue from fed normal rats, although the magnitude of this effect is not as great as that produced by insulin *in vitro*[12,13] To facilitate visual presentation, these data have been presented as bar graphs indicating mean values for control and hormone treated tissues. However, in each of these experiments one epididymal fat pad from each animal served as a control for the other. These are paired experiments whose significance can be judged from statistical analysis of the difference between the control and hormone stimulated pad. The increased utilization of glucose stimulated by the addition of growth hormone *in vitro* is not accompanied by an increased incorporation of glucose carbon into long chain fatty acids (Fig. 3), and fatty acid synthesis from glucose was actually decreased in most experiments.[12] The possible physiologic significance of these *in vitro* effects of growth hormone and prolactin with regard to the mechanism of their action in the intact animal will not be discussed at this time, but they will be used as laboratory tools to explore the relationship between glucose metabolism and the synthesis of fatty acid with particular regard to the action of insulin in adipose tissue.

As shown in Figure 4, insulin *in vitro* has no consistent effect on fatty acid synthesis from acetate-1–C^{14} or pyruvate-2–C^{14}, known precursors of acetyl-CoA, when these substrates are present alone in the medium.[7] If unlabeled glucose is present in the medium, in addition to labeled acetate or pyruvate, a definite stimulation by insulin of fatty acid synthesis from acetate or pyruvate is observed.[7] From these data it would appear that the effect of insulin on the synthesis of fatty acid in adipose tissue

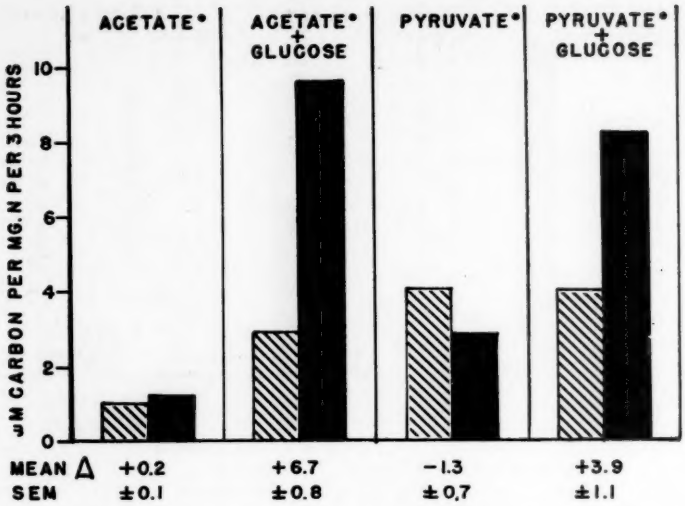

FIG. 4. Effect of insulin *in vitro* on fatty acid synthesis from acetate-1–C^{14} and pyruvate-2–C^{14} in rat adipose tissue. In the experiments with acetate the values represent μM of carbon-1 of acetate-1–C^{14} incorporated into total petroleum ether extractable lipids per mg. tissue nitrogen, which in this instance have been shown to represent primarily long chain fatty acid. The values listed for the experiments with pyruvate represent μM of carbon-2 of pyruvate-2–C^{14} incorporated into long chain fatty acid, determined as such, per mg. tissue nitrogen. Krebs bicarbonate buffer pH 7.4. Three hour incubation. Tissues paired. Striped bars indicate values for controls. Solid bars values for adipose tissue to which insulin (0.1 unit per ml.) was added *in vitro*.

Substrates Present in Medium

Acetate* − sodium acetate-1–C^{14} (60 μM per ml.)
Acetate* + glucose-sodium acetate-1–C^{14} (60 μM per ml.) plus unlabeled glucose (10 μM per ml.).
Pyruvate* − sodium pyruvate-2–C^{14} (40 μM per ml.)
Pyruvate* + glucose-sodium pyruvate-2–C^{14} (40 μM per ml.) plus unlabeled glucose (10 μM per ml.)

is related to its primary effect on glucose metabolism, and not to an effect on a specific step in fatty acid synthesis.

The recent work of Wakil[14] and Brady,[15] which indicates that malonyl CoA is an intermediate in lipogenesis in the liver of pigeons, prompted the study of the metabolism of malonate and acetaldehyde by adipose tissue from fed normal rats. This was to determine whether or not these substrates are incorporated into long chain fatty acid in adipose tissue, and if so, whether this incorporation is influenced by glucose and insulin.

As shown in Figure 5, when adipose tissue from fed normal rats is incubated with malonate-2–C^{14} in a concentration of 10μ M per ml. the appearance of carbon-2 of malonate in CO_2 can be demonstrated but its incorporation into fatty acid is not significant. Glucose stimulates the oxidation of carbon-2 of malonate to CO_2 as well as its incorporation into long chain fatty acid. Insulin *in vitro* stimulates fatty acid synthesis from malonate-2–C^{14} when unlabeled glucose is present in the medium. Insulin *in vitro* has no effect on the incorporation of malonate carbon into fatty acid unless glucose is present in the medium.

When adipose tissue from fed normal rats was incubated with malonic acid-2–C^{14} in a concentration of 20 μM per ml., twice that used in the preceding experiments, carbon-2 of malonate did not appear in either CO_2 or fatty

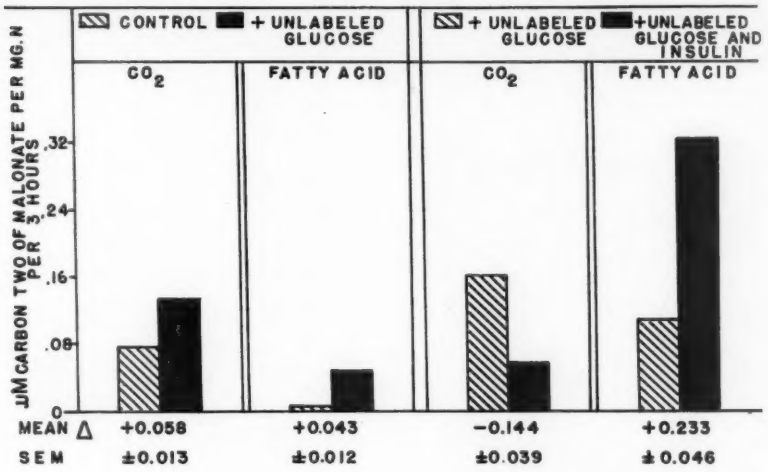

FIG. 5. Effect of unlabeled glucose and glucose plus insulin on the metabolism of malonic acid-2-C^{14} by rat adipose tissue. Incubation carried out in Krebs bicarbonate buffer containing malonic acid-2-C^{14} (10 μM per ml.); unlabeled glucose when present (10 μM per ml.); and insulin, when present (0.1 unit per ml.). Tissues paired to provide a control from the same animal for each experiment.

acid. At this higher concentration of substrate neither the addition of glucose nor of glucose plus insulin increased the appearance of malonate carbon in CO_2 or fatty acid. It appeared that at 20 μM per ml. malonate was interfering with the respiration of adipose tissue. The oxidation of succinate-2-C^{14} to CO_2 by adipose tissue from fed normal rats is not inhibited by malonic acid in concentrations as high as 10 μM per ml. (Fig. 6). At 20 μM per ml. malonic acid produces a marked inhibition of succinate oxidation and accounts for the inability to demonstrate the incorporation of malonate into fatty acid by adipose tissue at higher concentrations of substrate.

Adipose tissue from fed normal rats can incorporate the carbon of acetaldehyde-1, 2-C^{14} into long chain fatty acid (Fig. 7), but in the intact tissue acetate-1-C^{14} appears to be a better substrate for fatty acid synthesis. Glucose stimulates the incorporation of acetaldehyde-1,2-C^{14} into long chain fatty acid in the adipose tissue of the rat and, as shown in Figure 8, insulin *in vitro* stimulates the incorporation of acetaldehyde carbon into fatty acid when glucose is present in the medium. As with malonate, insulin has no consistent effect on the incorporation of acetaldehyde carbon into long chain fatty acid unless glucose is also present in the medium. Thus the effect of insulin on fatty acid synthesis from malonate and acetaldehyde also appears to be related to its primary effects on glucose metabolism and not on a specific step in fatty acid synthesis.

At least two and perhaps three pathways for the metabolism of glucose are present in adipose tissue.[12,13,16] Although the methods for estimating what proportion of the total glucose utilized traverses an individual pathway are inadequate, certain patterns can be discerned and gross changes documented. Both the Embden-Meyerhof and phosphogluconate oxidative pathways are operative in the epididymal fat pad.[12,16,17] Calculations based upon the incorporation of carbon atoms 1 and 6 of glucose into long chain fatty acid in adipose tissue from fed normal rats (Table II) suggest that approximately equal proportions of glucose carbon atom-6 isolated in fatty acid come from glucose molecules which traverse glycolytic and non-glycolytic pathways. Insulin *in vitro* stimulates fatty acid synthesis from glucose but does not alter these proportions.[16] These data cannot be extrapolated to give an estimate of what

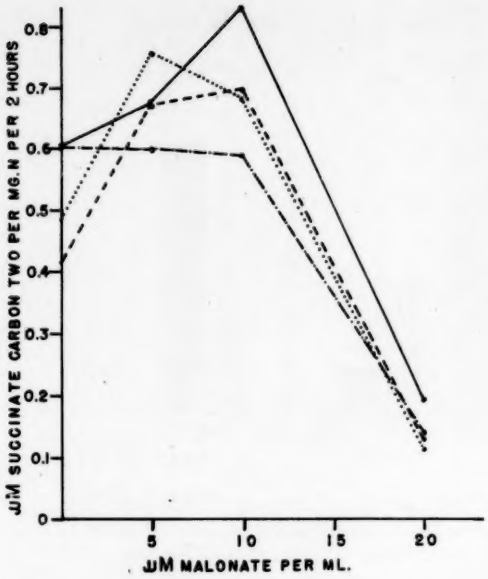

FIG. 6. Effect of malonate on the conversion of succinate-2–C^{14} to carbon dioxide by rat adipose tissue. Each epididymal fat pad was divided by a single cut into its two horns, thus providing four pieces of adipose tissue from the same animal. Incubation was carried out in Krebs bicarbonate buffer, pH 7.4, containing sodium succinate-2–C^{14} (10 μM per ml.). One piece of tissue served as a control; to the others unlabeled malonic acid was added in final concentrations of 5, 10 and 20 μM per ml. Points represent the μM of carbon-2 of succinate oxidized to CO_2 per mg. of nitrogen by each piece of tissue. The lines join the points representing the four pieces of tissue from one animal.

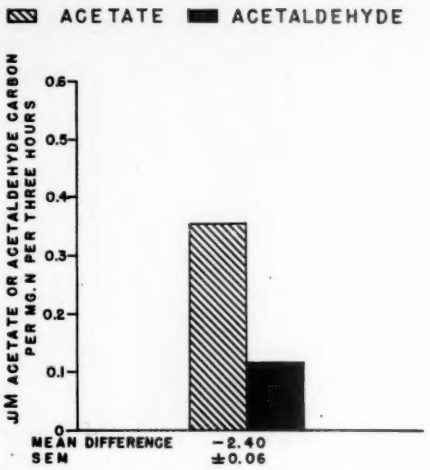

FIG. 7. Comparison of the incorporation of acetate-1–C^{14} and acetaldehyde-1,2–C^{14} into fatty acid by normal fed rat adipose tissue. Tissues paired to compare the incorporation of acetate and acetaldehyde carbon into long chain fatty acid by adipose tissue from the same animal. Incubation carried out in Krebs bicarbonate buffer pH 7.4 containing acetate-1–C^{14} (60 μM per ml.) or acetaldehyde-1,2–C^{14} (60 μM per ml.). The specific activities of the acetate and acetaldehyde carbons were approximately the same.

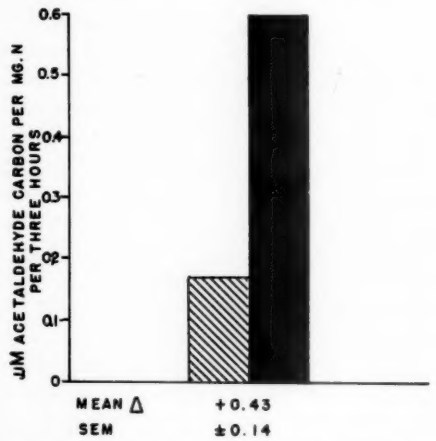

FIG. 8. Effect of insulin *in vitro* on the incorporation of acetaldehyde-1,2–C^{14} into fatty acid by rat adipose tissue. Tissues paired. Incubation carried out in Krebs bicarbonate buffer pH 7.4 containing acetaldehyde-1,2–C^{14} (60 μM per ml.) and unlabeled glucose (10 μM per ml.). Striped bar represents mean value for the incorporation of acetaldehyde carbon into long chain fatty acids in controls. Solid bar represents mean value for fat pads to which insulin (0.1 unit per ml.) was added *in vitro*.

percentage of total glucose utilization traverses a given pathway. The data on CO_2 production in these same experiments suggested increased glucose utilization by the phosphogluconate oxidative pathway.

A system has been devised which permits the hourly comparison of the production of $C^{14}O_2$ by paired epididymal fat pads from fed normal rats incubated with glucose-1–C^{14} and glucose-6–C^{14} over a four hour period.[12] As seen in Figure 9, when adipose tissue from the rat is incubated without added hormone the production of CO_2 from carbon-1 of glucose tends to exceed the production from carbon-6 throughout the four hour period of incubation. These observations confirm our earlier experiments[16] and those of Milstein[17] which indicate

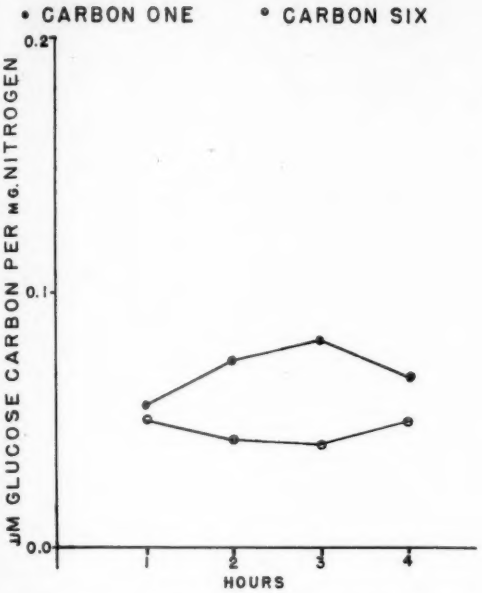

FIG. 9. CO_2 production from carbon atoms one and six of glucose by rat adipose tissue. Incubation carried out in Krebs-Ringer phosphate buffer pH 7.4. Concentration of glucose-1–C^{14} or glucose-6–C^{14} was 20 μM per ml. Tissues paired so as to compare the metabolism of carbon-1 and carbon-6 of glucose in tissue from the same animal. No hormone was added.

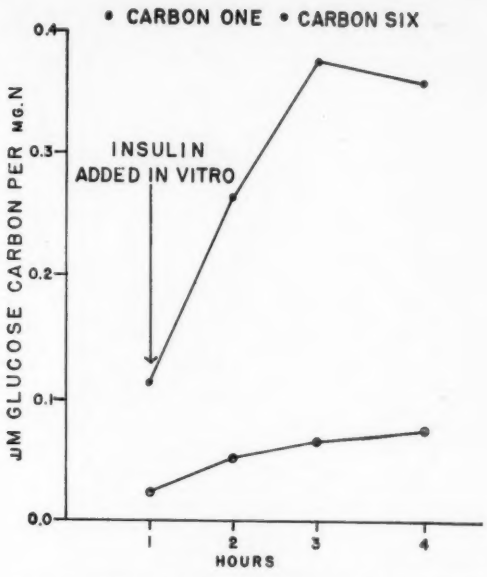

FIG. 10. Effect of insulin *in vitro* on CO_2 production from carbon atoms one and six of glucose by rat adipose tissue. Incubation carried out in Krebs-Ringer phosphate buffer, pH 7.4. Concentration of glucose-1–C^{14} or glucose-6–C^{14} was 20 μM per ml. Tissues paired. Final concentration of insulin, 0.1 unit per ml. added *in vitro* to both incubation vessels at the end of a one hour control period. (Glucagon-free insulin provided by Eli Lilly Research Laboratories.)

that the phosphogluconate oxidative pathway participates in the utilization of glucose in adipose tissue from fed rats. Moreover, high levels of glucose-6–phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in this tissue can be demonstrated.[18]

TABLE II
Effects of Insulin Added *in Vitro* on the Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} by Rat Adipose Tissue*

	Glucose to CO_2		Glucose to Fatty Acids	
	C_1	C_6	C_1	C_6
Control	0.92 ± .06	0.24 ± .10	0.26 ± .02	0.55 ± .05
Insulin	6.36 ± .46	0.33 ± .02	3.14 ± .50	6.39 ± 1.02

* Incubation of paired epididymal fat pads from fed, normal rats in Krebs bicarbonate buffer, pH 7.4. One pad from each animal incubated with glucose-1–C^{14} and the other with glucose-6–C^{14} (both in concentration of 20 μM per ml.). Three hour incubation. All values expressed as μM of carbon-1 or carbon-6 incorporated into CO_2 or long chain fatty acid per mg. of tissue nitrogen.[16]

Paired fat pads were incubated for one hour without the addition of hormone to obtain control values for the relative rates of CO_2 production from carbons-1 and 6 of glucose, and insulin was then added to both vessels (Fig. 10). There was a marked increase in the production of CO_2 from carbon-1 and a less striking increase in the oxidation of carbon-6.[12] This representative experiment supports the participation of the phosphogluconate oxidative pathway in the increased metabolism of glucose stimulated by the addition of insulin *in vitro*.

Figure 11 shows a similar experiment in which prolactin is added *in vitro* at the end of a one hour control period.[13] Prolactin stimulates the oxidation of carbon-1 of glucose to CO_2 relative to carbon-6 in a manner which resembles the effect of the addition of insulin in this same preparation. The consistent isolation of greater quantities of carbon-1 of

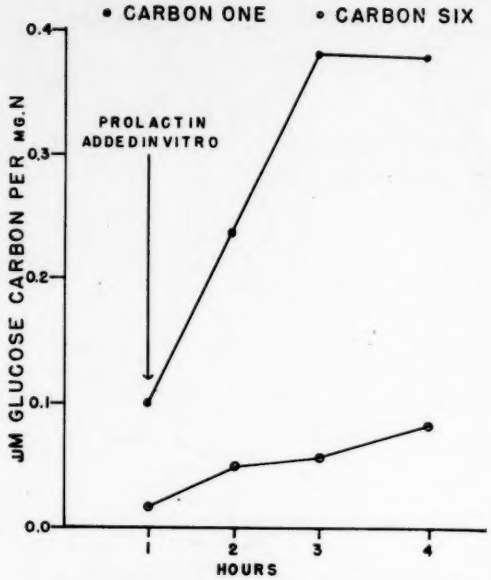

FIG. 11. Effect of ovine prolactin *in vitro* on CO_2 production from carbon atoms one and six of glucose by rat adipose tissue. Incubation carried out in Krebs-Ringer phosphate buffer, pH 7.4. Concentration of glucose-1-C^{14} or glucose-6-C^{14} was 20 μM per ml. Tissues paired. Ovine prolactin (Armour Laboratories, gift of NIH Endocrinology Study Section) added *in vitro* in a final concentration of 1000 gamma per ml. to both incubation vessels at the end of a one hour control period.

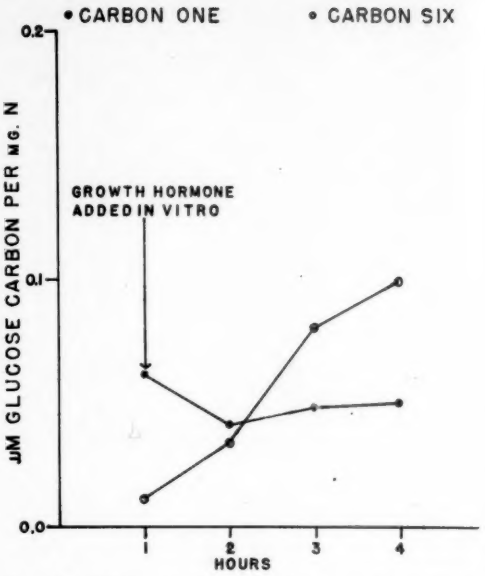

FIG. 12. Effect of bovine growth hormone *in vitro* on CO_2 production from carbon atoms one and six of glucose by rat adipose tissue. Incubation carried out in Krebs-Ringer phosphate buffer, pH 7.4. Concentration of glucose-1-C^{14} or glucose-6-C^{14} 20 μM per ml. Tissues paired. Bovine growth hormone (Wilhelmi, gift of NIH Endocrinology Study Section) added *in vitro* in a final concentration of 1000 gamma per ml. to both incubation vessels at the end of a one hour control period.

glucose in CO_2 than of carbon-6 would support the participation of the phosphogluconate oxidative pathway in the increased utilization of glucose stimulated by prolactin *in vitro*.

If bovine growth hormone is added *in vitro* at the end of a one hour control period to paired epididymal fat pads, incubated in differentially labeled glucose (Fig. 12), there is, within two hours, a marked increase in the rate of production of CO_2 from carbon-6 both absolute and relative to carbon-1.[12] This representative experiment indicates that bovine growth hormone stimulates glucose metabolism in the adipose tissue of the rat in a manner which results in the more rapid oxidation of carbon-6 to CO_2 than of carbon-1. At least two interpretations of this finding are possible. First, the uronic acid pathway[19] is operative in the adipose tissue of the rat when stimulated by growth hormone *in vitro*. Secondly, and considered less likely, growth hormone stimulates glucose utilization in adipose tissue primarily through the Embden-Meyerhof pathway and the more rapid appearance of carbon-6 than of carbon-1 in CO_2 results from the incomplete equilibration of the trioses derived from glucose.

The uronic acid pathway for glucose metabolism, adapted from Horecker and Hiatt,[19] is represented in Figure 13. If this pathway were operative in adipose tissue and glucuronic acid-6-C^{14} gained entrance to the cell, it would yield labeled CO_2 and unlabeled L-xylulose. The glucose-6-phosphate resynthesized from L-xylulose would be unlabeled as would the products derived from its further metabolism. When adipose tissue from fed normal rats was incubated *in vitro* with glucuronic acid-6-C^{14} in the absence of added hormone (Table III) the oxidation of carbon-6 of glucuronic acid to CO_2 could be demon-

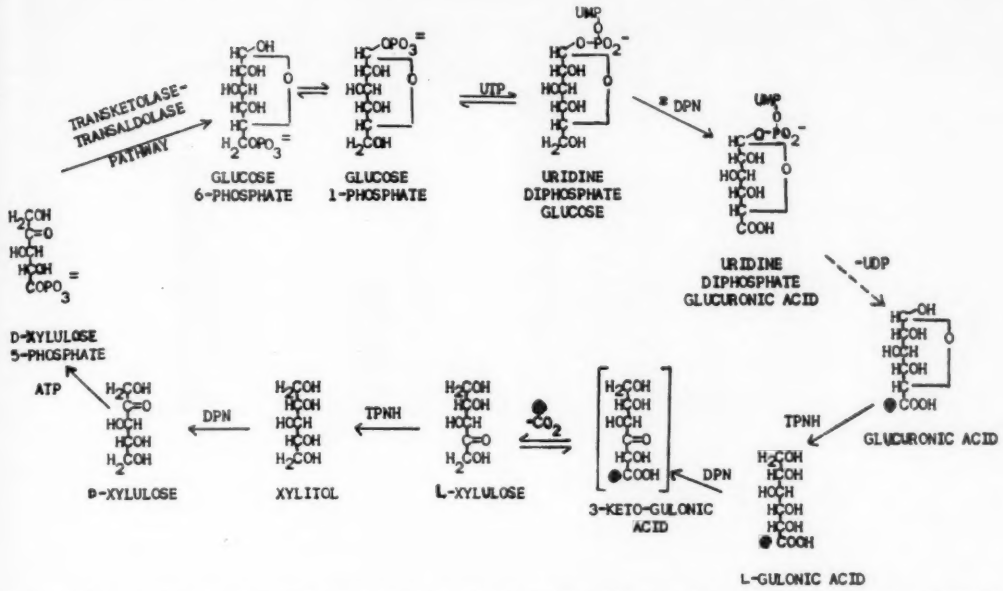

FIG. 13. The uronic acid pathway of carbohydrate metabolism adapted from the formulation of Horecker and Hiatt.[1]

strated. Moreover, there was no incorporation of carbon-6 of glucuronic acid-6-C^{14} into fatty acid. This excludes the possibility that the labeled glucuronic acid was contaminated with glucose, or that glucuronic acid was in some way converted to glucose before losing its sixth carbon. The data support the existence of the uronic acid pathway in adipose tissue, and suggest that the addition of growth hormone *in vitro* stimulates glucose metabolism by this pathway as well as by the glycolytic pathway. It is of importance, with regard to the effect of glucose metabolism on the synthesis of fatty acid, to point out that the metabolism of glucose by the uronic acid pathway results in the oxidation of triphosphopyridine nucleotide reduced (TPNH) to triphosphopyridine nucleotide (TPN),[19] whereas glucose utilization via the phosphogluconate oxidative pathway leads to the production of TPNH which is essential for fatty acid synthesis. Thus the phosphogluconate oxidative pathway does not participate to the same degree in the increased metabolism of glucose stimulated by the addition of growth hormone to adipose tissue, as it does when either insulin or prolactin are the stimulating agents.

TABLE III
Glucuronic Acid-6-C^{14} Metabolism by Rat Adipose Tissue*

Animal	CO_2	Fatty Acid
1	.046	.000
2	.070	.000
3	.049	.000
4	.130	.000
5	.071	.000
6	.084	.000
7	.072	.000
Mean	.075	.000
Standard error	± .011	...

* Incubation of adipose tissue in Krebs-bicarbonate buffer, pH 7.4, containing glucuronic acid-6-C^{14} (5 μM per ml.): three-hour incubation. All values represent μM of carbon-6 of glucuronic acid incorporated into CO_2 or long chain fatty acid per mg. of tissue nitrogen.

It has been shown that insulin and prolactin stimulate glucose utilization by the adipose tissue of the rat, and that in each instance the increased glucose metabolism, with respect to CO_2 production, appears to be channeled through the enzymatic pathways in a similar and characteristic manner. It is interesting to observe (Fig. 14), therefore, that prolactin *in vitro* affects fatty acid synthesis from acetate

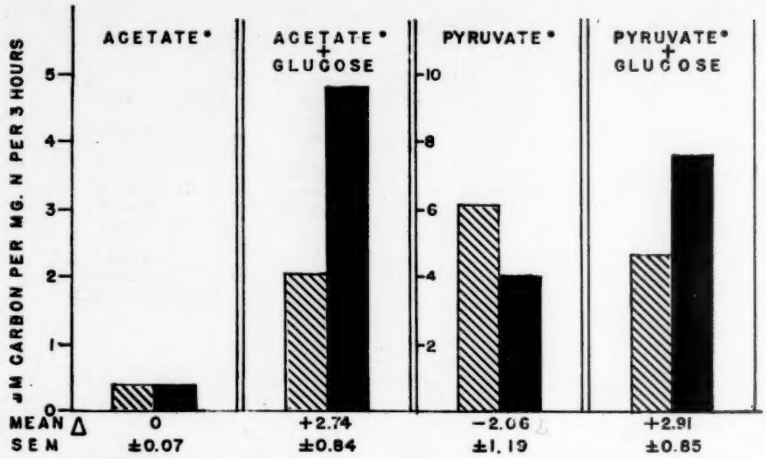

FIG. 14. Effect of ovine prolactin *in vitro* on fatty acid synthesis from acetate-1–C^{14} in rat adipose tissue. All values expressed as μM of carbon-1 or acetate-1–C^{14} or carbon-2 of pyruvate-2–C^{14} incorporated into long chain fatty acid per mg. of adipose tissue nitrogen. Three hour incubation. Krebs bicarbonate buffer, pH 7.4. Tissues paired. Striped bars indicate values for controls. Solid bars indicate values for adipose tissue to which prolactin (1000 gamma per ml.) was added *in vitro*.

Acetate• −medium contained sodium acetate-1–C^{14} (60 μM per ml.)

Acetate• + glucose-medium contained unlabeled glucose (10 μM per ml.) in addition to acetate-1–C^{14} (60 μM per ml.)

Pyruvate• −medium contained sodium pyruvate-2–C^{14} (40 μM per ml.) .

Pyruvate• + glucose-medium contained unlabeled glucose (10 μM per ml.) in addition to pyruvate-2–C^{14} (40 μM per ml.)

and pyruvate in adipose tissue in a manner which resembles that of insulin. Prolactin *in vitro* has no effect on fatty acid synthesis from either of these two precursors of acetyl-CoA when they are present alone in the medium. However, in the presence of unlabeled glucose a definite stimulation of synthesis of fatty acid from acetate-1–C^{14} and pyruvate-2–C^{14} is observed. The effects of prolactin on fatty acid synthesis in adipose tissue thus appear to be secondary to its effects of glucose metabolism. Moreover, the increased glucose metabolism, stimulated by prolactin *in vitro*, resembles that stimulated by insulin and, on the basis of CO_2 data, suggests active glucose utilization via the phosphogluconate oxidative pathway.

The problem of the effects of growth hormone on fatty acid synthesis in adipose tissue is more complex. As shown in Figure 15, growth hormone has a slight inhibitory effect on fatty acid synthesis from acetate or pyruvate when these substrates are present alone in the medium. Moreover, if, in addition to either acetate-1–C^{14} or pyruvate-2–C^{14}, unlabeled glucose is present in the medium, growth hormone markedly decreases the incorporation of acetate-1–C^{14} and pyruvate-2–C^{14} into long chain fatty acid as compared with the glucose stimulated control. Growth hormone *in vitro* stimulates glucose uptake and oxidation to CO_2 by rat adipose tissue, but the phosphogluconate oxidative pathway does not contribute to a major degree to this increased CO_2 production. This increased utilization of glucose does not result in an increased incorporation of glucose carbon into fatty acid, nor does it stimulate fatty acid synthesis from known precursors of acetyl-CoA such as acetate and pyruvate.

It is important to note (Fig. 16) that if insulin is added *in vitro* to one of a pair of epididymal fat pads, both pads being incubated with glucose-U–C^{14} in the presence of growth hormone, insulin brings about a further increase

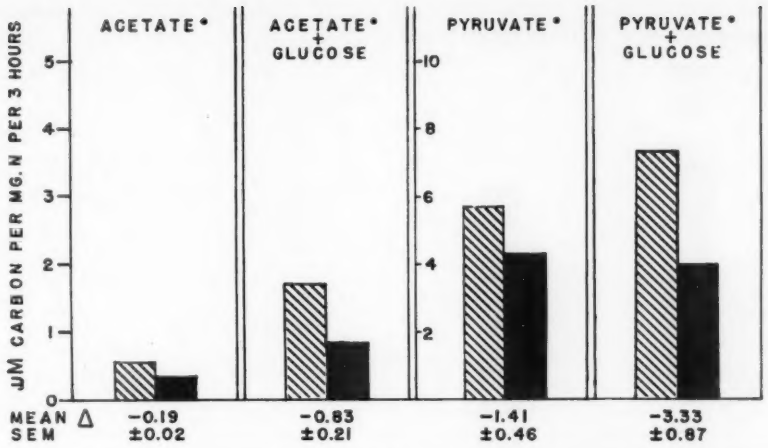

FIG. 15. Effect of bovine growth hormone *in vitro* on fatty acid synthesis from acetate-1–C^{14} and pyruvate-2–C in rat adipose tissue. All values expressed as μM of carbon-1 of acetate-1–C^{14} or carbon-2 of pyruvate-2–C^{14} incorporated into long chain fatty acid per mg. of adipose tissue nitrogen. Krebs bicarbonate buffer pH 7.4. Three hour incubation. Tissues paired. Striped bars indicate values for controls. Solid bars indicate values for adipose tissue to which growth hormone (1000 gamma per ml.) was added *in vitro*.

Acetate* —medium contained sodium acetate-1–C^{14} (60 μM per ml.)

Acetate* + glucose-medium contained unlabeled glucose (10 μM per ml.) in addition to acetate-1–C^{14} (60 μM per ml.)

Pyruvate* —medium contained sodium pyruvate-2–C^{14} (40 μM per ml.)

Pyruvate* + glucose-medium contained unlabeled glucose (10 μM per ml.) in addition to pyruvate-2–C^{14} (40 μM per ml.)

in the production of CO_2 from glucose, and the incorporation of glucose into long chain fatty acid is markedly increased as compared to the pad incubated with growth hormone alone.

If paired epididymal fat pads are incubated with glucose-1–C^{14} and glucose-6–C^{14} in a procedure which permits the hourly comparison of the production of CO_2 from carbon atoms 1 and 6 of glucose (Fig. 17), and growth hormone is added *in vitro* to both vessels at the beginning of the incubation period, within two hours a reversal of the normal control pattern and the appearance of more carbon-6 in CO_2 than of carbon-1 is observed.[12] If at the end of two hours insulin is added to both vessels (Fig. 17), there is an abrupt increase in the production of CO_2 from carbon-1, and an abrupt change in the relative proportions of carbons 1 and 6 of glucose appearing in CO_2, so that the amount of carbon-1 appearing in CO_2 approaches the amount of carbon-6 isolated in the same period. No dogmatic interpretation of these experiments can be made. However, they suggest that insulin not only increases the total glucose utilized, but also alters the proportions of the total glucose oxidized to CO_2 which traverse the individual enzymatic pathways in adipose tissue. It is of note that this increased utilization of glucose via the phosphogluconate oxidative and Embden-Meyerhof pathways is associated with an increase in the synthesis of fatty acid from glucose. It would thus appear that hormonal regulation of the synthesis of fatty acid in adipose tissue might be achieved not only by the control of glucose uptake, but also by alterations in the specific pathways of glucose metabolism operative at any given time.

SUMMARY

The regulation of fatty acid synthesis in adipose tissue has been studied *in vitro* using the paired epididymal fat pads of the rat. Conditions which impair the utilization of glucose in this tissue, such as starvation or the

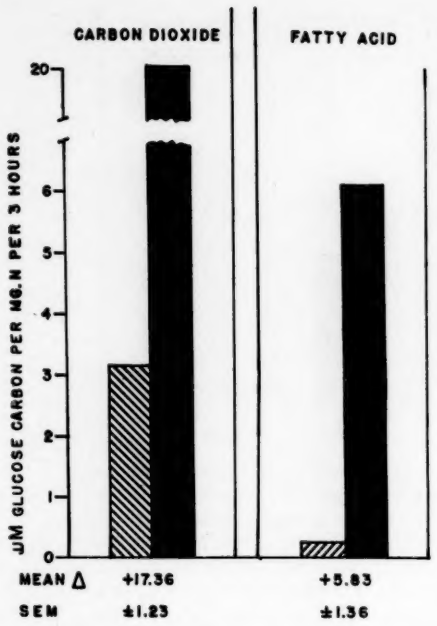

FIG. 16. Effects of insulin and bovine growth hormone *in vitro* on metabolism of glucose-U-C^{14} by rat adipose tissue. Incubation carried out in Krebs bicarbonate buffer, pH 7.4, containing uniformly labeled glucose-C^{14} (20 μM per ml.). Tissues paired. Bovine growth hormone (1000 gamma per ml.) added to both epidiymal fat pads; insulin (0.1 unit per ml.) added *in vitro* in addition to growth hormone to one pad of each pair. Striped bars indicate values for pads incubated with growth hormone alone. Solid bars indicate values for the pads to which insulin was added in addition to growth hormone.

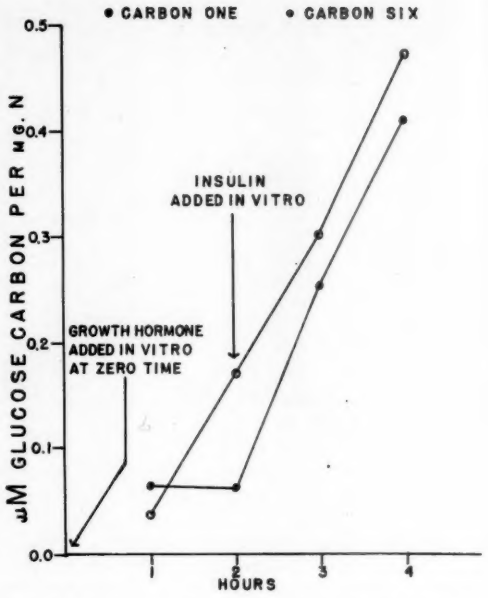

FIG. 17. Effects of bovine growth hormone and insulin *in vitro* on CO_2 production from carbon atoms one and six of glucose by rat adipose tissue. Incubation carried out in Krebs-Ringer phosphate buffer, pH 7.4. Concentration of glucose-1-C^{14} or glucose-6-C^{14} was 20 μM per ml. Tissues paired. Bovine growth hormone (1000 gamma per ml.) added *in vitro* to both vessels at the start of the incubation period. Insulin (0.1 unit per ml.) added *in vitro* to both vessels at the end of two hours.

induction of alloxan diabetes, virtually abolish fatty acid synthesis in adipose tissue. Insulin *in vitro* stimulates fatty acid synthesis from glucose in tissue from fed normal animals, and restores lipogenesis in adipose tissue from starved rats or rats with alloxan diabetes. Insulin also stimulates the incorporation of carbon from acetate, acetaldehyde, malonate and pyruvate into fatty acid but only in the presence of glucose. The effects of insulin on fatty acid synthesis from these four substrates thus appears to be secondary to its effects on carbohydrate metabolism in adipose tissue. The Embden-Meyerhof and phosphogluconate oxidative pathways are operative in adipose tissue, and evidence for the presence of the uronic acid pathway is presented. The phosphogluconate oxidative pathway, an important source of TPNH which is required for fatty acid synthesis, participates in the increased oxidation of glucose to CO_2 stimulated by insulin or ovine prolactin *in vitro*. This increased utilization of glucose is accompanied by increased fatty acid synthesis from glucose, and stimulates lipogenesis from acetate and pyruvate carbon in this tissue. The phosphogluconate oxidative pathway does not participate to the same extent in the increased production of CO_2 from glucose which follows the addition of bovine growth hormone *in vitro*. Growth hormone does not increase the incorporation of glucose carbon into long chain fatty acid, nor does it stimulate lipogenesis from acetate or pyruvate in the presence or absence of glucose. Evidence is presented which suggests that hor-

monal regulation of fatty acid synthesis in adipose tissue might be achieved not only by the control of glucose uptake, but also by alterations in the specific pathways of glucose metabolism operative at any given time.

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We wish to express our appreciation for the expert technical and experimental assistance of Miss Mary Ann Holzinger, Miss Ann Stetser and Miss Mary Lou Sears.

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DISCUSSION

DR. MAX KLEIBER (*Davis, California*): May I be permitted to add confirmation of your findings *in vitro* by results in the intact normal cow. When we inject labeled acetate into one quarter of the udder which is conveniently separated from the other quarter, we find that the higher fatty acids of the butter fat are much more highly labeled than those originating from the quarter which was not injected at the same time. This can only mean that the synthesis has been taking place in that quarter. That offers the possibility of testing in the intact animal, where the synthesis takes place.

DR. C. N. H. LONG (*New Haven, Connecticut*): How pure is this prolactin? Have you any idea whether it is the lactogenic agent itself, or any contamination?

We now have adrenotrophic agents which can act without the presence of the adrenals. How far have you explored the possibility that it is prolactin that is doing this, and not something that is being carried along with it.

DR. WINEGRAD: The preparation which we used is from bovine prolactin distributed by the National Institutes of Health. Dr. Wilhelmi prepared our growth hormone preparation which was also provided by the National Institutes of Health.

When we originally experimented with this material, we made control studies, in which we employed bovine serum albumin, and we could not obtain consistent results. We also used commercial ACTH and were unable to obtain effects similar to those observed with growth hormone, although I am told that other preparations of ACTH may do this.

We specifically wanted to avoid any implication that the observed effects of growth hormone and prolactin *in vitro* on the metabolism of glucose by adipose tissue explain the action of these hormones on their usual target tissues, but rather to use these effects as laboratory tools for the time being.

DR. ALBERT RENOLD (*Boston, Massachusetts*): I want to comment on the most challenging part of Dr. Winegard's presentation. I would disagree with his interpretation that there must be an effect of insulin beyond its effect on the entry of glucose into cells. In like manner, is the effect of growth hormone on glucose entry a primary one? Could it not be an indirect effect of some intracellular metabolic action?

DR. WINEGRAD: Growth hormone *in vitro* does not stimulate glucuronic acid metabolism by adipose tissue from normal fed rats. If the action of growth hormone were on any part of the uronic acid cycle, from glucuronic acid on, one would anticipate that such would be the case.

We are in the process of investigating whether or not growth hormone affects earlier steps in this cycle, but to date the evidence would suggest that it does not.

Dr. Wick, in studies with 6-fluoroglucose and 2-deoxyglucose in this tissue, has shown that there are probably two pathways for glucose entry into the adipose tissue cell prior to the hexokinase step.

As long as one assumes that there has to be only one site of glucose entry, for which we have no evidence, then I can see your objection. But in view of the increasing evidence that there is compartmentation of function, I would think that this is more likely to be the explanation.

DR. RACHMIEL LEVINE (*Chicago, Illinois*): Dr. Winegrad, would it not be helpful, in distinguishing the two points of entry, to see whether growth hormone or prolactin increases the entry into adipose tissue, or

equilibrates C^{14}-marked galactose, xylose, or any of the nonmetabolizable sugars, which insulin does?

DR. WINEGRAD: We have not made such studies. After I left Dr. Renold's laboratory, Dr. Cahill was interested in pursuing just this point. Perhaps he might care to comment on this.

DR. GEORGE F. CAHILL, JR. (*Boston, Massachusetts*): We never got to the experiments, the main reason being that adipose tissue is only some 23 per cent water, and of the 23 per cent, only 4 per cent is intracellular, and to pick up any penetrability problem would be difficult.

I want to point out one thing on the study of the effects of growth hormone on the uronic acid pathway. The time studies were performed in phosphate buffer. We have repeated the studies in bicarbonate buffer and get perfect linearity. This delayed time may be another one of the problems of buffer with which we have been beset many times, before.

Why should adipose tissue have a uronic acid pathway?

DR WINEGRAD: I would think it possible that if this tissue were capable of synthesizing polysaccharides–mucopolysacchrides, for example–it would be perfectly reasonable to have a system for making uridine diphospho-glucuronic acid, since this, apparently, is the way in which glucuronic acid is incorporated into mucopolysaccharides.

The other thing which I do not think your comment can particularly explain is the fact that the tissue can use glucuronic acid.

DR. ESTELLE RAMEY (*Washington, D. C.*): I would like to make a comment on the buffer that Dr. Cahill mentioned. Our experience with both adipose and diaphragm tissue, *in vitro*, is that any hormones we use, particularly the steroids and growth hormone, are far more effective, *in vitro*, in a phosphate buffer than they are in a bicarbonate buffer. There are many times when we get no effect of hormone whatever, *in vitro*, in the more physiologic buffer, namely, the bicarbonate buffer, but we can obtain the effects in the non-physiologic, or relatively non-physiologic, buffer, which is the phosphate buffer.

In going through the literature, we have accumulated a good deal of evidence from other laboratories that this is not uncommon—that it is the phosphate buffer which makes possible many *in vitro* experiments with several of the hormones.

DR. WINEGRAD: You have misinterpreted Dr. Cahill's comment. All of our experiments were done in bicarbonate buffer with the exception of the time studies. It is impossible to collect carbon dioxide at hourly intervals in a bicarbonate buffer, because you have to kill your tissue in order to do so.

On the other hand, we did carry out studies on the relative incorporations of carbons 1 and 6 of glucose into carbon dioxide in bicarbonate buffer, and we reported these experiments in detail in the *Journal of Biological Chemistry*, 234: 1922, 1959.

The reason for devising this system in which we use the phosphate buffer for hourly collection was that we found an alteration in the pattern of carbons 1 and 6 of glucose into CO_2 when we added growth hormone to adipose tissue incubated in bicarbonate buffer. Under those circumstances there were equal quantities of carbons 1 and 6, isolated in CO_2.

We also studied the relative incorporation of carbon 1 of glucose-1–C^{14}, as compared with carbons 1 and 6 of glucose-1-6–C^{14}, and found that about 50 per cent of the carbon seemed to be coming from carbon 1 and 50 per cent from carbon 6.

At that point we believe that what we were doing with growth hormone was merely inhibiting the phosphogluconate oxidative shunt. In a phosphate buffer, carbon 6 came out more rapidly than carbon 1. Sometimes this also happens in a bicarbonate buffer.

At the time we could not explain these results, but as the work which was done on the various enzymatic steps of the glucuronic acid pathway was reported, we were led to conclude that the glucuronic acid pathway must be present in this tissue.

I think this is an answer to the implication that all these effects are due to a phosphate buffer, since this was not used in our studies except for time experiments.

DR. LEVINE: Dr. Winegrad, what effect has your work on growth hormone, prolactin, and now Dr. Cahill's on adrenalin on the insulin assay in an *in vitro* tissue, merely on the uptake of sugar?

DR. WINEGRAD: I think Dr. Renold should be responsible for the answer to that question.

DR. RENOLD: The relative non-specificity of a bioassay system is something with which we should learn to live because this is by no means limited to insulin. This extends to all types of bioassay. Oxytocin and vasopressin, particularly well clarified chemical entities, share this in common: there is nothing that one of these hormones does that the other does not do also. But they do it at different concentrations.

Although I have no insight into the actual levels of prolactin to be expected *in vivo*, I would hope that these effects of prolactin would not be present in adipose tissue in the concentrations usually present *in vivo*, let's say in a non-lactating male or perhaps even in a lactating female.

This is something that is innate in the nature of a bioassay. Whenever a question of this kind arises, one has to rule it out in each specific instance whether this is insulin or not, by purification or whatever other procedure one has to apply. These things demonstrate completely that there is the possibility of non-specificity, and I do not think there is any reason why they should not.

DR. WINEGRAD: The magnitude of the effect of prolactin certainly does not approach that of insulin in the same tissue. Moreover, in adipose tissue from rats with alloxan diabetes, although one can show consistently an effect of prolactin on glucose oxidation to CO_2, this increase is not marked and is not apparently sufficient to restore fatty acid synthesis in this tissue.

Perhaps if your assay were altered so as to be done on rats with alloxan diabetes, this interference with prolactin could be avoided completely.

DR. RENOLD: It does bring out one general point, that frequently we should consider matters of dosage. Often in studies of hormones, *in vitro*, we deal in qualitative effects, and the matter of the dosage at which the effect is obtained is crucial.

The effect of insulin on mammary gland is an example. We have effects similar to those which we might have with prolactin. They occur at concentrations one hundred to a thousand times those which affect adipose tissue.

It is more likely that the effect of insulin on adipose tissue is the biologically significant one, but this is a matter of interpretation.

DR. R. H. WILLIAMS (*Seattle, Washington*): Dr. Winegrad, since the action of prolactin on the HMP was so slight in the diabetic animals, compared to the normal, I was wondering whether or not, the phenomenon here might not be comparable to what Dr. Narahara has shown with reference to the growth hormone effect on glucose uptake in muscle, where prolactin competes with insulin for the degrading enzyme, even in adipose tissue; and whether or not it might actually spare the degradation of insulin, so that in reality you would have predominantly an insulin effect?

DR. WINEGRAD: I would like to answer the question first with regard to growth hormone. There can be no confusion as to whether or not these effects are due to insulin bound to the tissue, since insulin *in vitro* will not reproduce the effect of growth hormone.

With regard to prolactin, we have been careful to point out that prolactin might well be inhibiting the degradation of insulin. (*Journal of Biological Chemistry*, 234: 3111, 1959.)

We are dealing here with the effects of bovine growth hormone and bovine prolactin in rat tissue. It has been well demonstrated that the pituitary hormones have a species specificity. Moreover, we are not studying the effects of these hormones in their usual target tissues. We are using them here merely to try to find out how glucose utilization influences fatty acid synthesis from acetate and pyruvate. However, it may well be that if one had good preparations of rat growth hormone or rat prolactin, one could demonstrate that these effects had some physiologic significance. Perhaps pituitary hormones produce their effect, in part, by increasing the glucose uptake of sensitive peripheral tissues, and by choosing what part of the cell this glucose happens to get into.

DR. SALIH WAKIL (*Durham, North Carolina*): What is the relative activity, or the relative synthesis of fatty acids, in the adipose tissue and in the homogenate of the adipose tissue? Will insulin exert this effect in the slices and not in the homogenate?

DR. WINEGRAD: Neither insulin nor growth hormone has any effects in a homogenate of adipose tissue. In a cell-free preparation we do not obtain any effect.

Dr. Shaw did not demonstrate any effect of growth hormone on, for example, glucose-6 phosphate dehydrogenase activity or on 6-phosphogluconate dehydrogenase activity when attempts were made to demonstrate this *in vitro*.

DR. WAKIL: If you compare the two relative activities, the homogenates may make fatty acids at their maximum and the addition of insulin may have no effect, since in the homogenate there may be no problem of permeability. I believe Dr. Levine thinks that the primary effect of this hormone is on permeability. Since there should be no problem of permeability and no insulin effect, fatty acid synthesis, or other activity would be at its height.

DR. LEVINE: It is fine with me if you can demonstrate it.

DR. SHAW: We made attempts to measure fatty acid synthesis in homogenates of adipose tissue. We cannot demonstrate any synthesis of long chain fatty acids with acetate as a substrate. We never fail if we use pyruvate. We can get a feeble but measurable synthesis with glucose.

With pyruvate, the level of incorporation closely approximates that which you can obtain with the whole tissue. With glucose, this is quite a different thing.

DR. LEVINE: In the homogenate it does not rise to any maximum which could not be raised in the whole tissue.

DR. CAHILL: Since with, specifically, labeled glucose, epinephrine, ACTH, probably thyroid stimulating hormone (TSH), and glucagon, there is exactly the same pattern of metabolism is there also an increase in the glucuronic acid pathway?

DR. WINEGRAD: In your experiments with epinephrine, has the carbon-6 of glucose actually come out more rapidly than carbon-1?

DR. CAHILL: Yes, it has.

DR. WINEGRAD: In time experiments, with one hour collections?

DR. CAHILL: ACTH works better. With epinephrine, in some animals, C-6 will be greater than C-1, using CO_2. With ACTH, most frequently it will. Therefore, does ACTH increase the glucuronic acid pathway?

DR. WINEGRAD: I have not studied it, but I would not be surprised, if what you say is true—that it might well increases the glucuronic acid pathway.

In most of your experiments you collected carbons 1 and 6 in total CO_2 production over a three hour period, and in most of those experiments you got equal quantities of carbons 1 and 6 in CO_2.

If with ACTH carbon 6 actually does come out faster, then I would be interested to see whether or not glucuronic acid-6-C^{14} is oxidized to CO_2. If it is, then, whether we like it or not, the third shunt is there, and there is not much we can do about it.

The Effect of Insulin on Adipose Tissue

RUSSELL J. BARRNETT, M.D.* AND ERIC G. BALL, PH.D.†

THE RESULTS of a combined biochemical and electron microscopic study of the changes produced by insulin in the adipose cells of the epididymal fat pad of the rat *in vitro* are reviewed.[1–3] These experiments followed the observations of Winegrad and Renold[4] who demonstrated that the addition of insulin to the epididymal fat pad of the rat, incubated in bicarbonate buffered media, markedly stimulates the net reactions 1 to 3 (Fig. 1) whereby glucose is converted to fat. Reaction 4, a true respiratory one, is not stimulated by the addition of insulin.

Reaction 1 = Glucose → 2 acetyl CoA + DPNH + 2 CO_2 + 2 P

Reaction 2 = Glucose + 1 P → pentose − PO_4 + 2 TPNH + CO_2

Reaction 3 = n(acetyl COA + 2n-2 (TPNH + DPNH) → Fatty acid

Reaction 4 = Glucose → 6 CO_2 + H_2O

FIG. 1. Unbalanced equation approximating the effect of insulin on the synthesis of lipid with a marked output of CO_2.

During the synthesis of fat from glucose, a marked excess of carbon dioxide output over oxygen consumption can be expected. The effect of insulin on the total exchange of gas in the epididymal fat pad was studied by Ball, Martin and Cooper[5] by means of the Warburg manometric apparatus. Paired pieces of epididymal fat pad, approximately 200 mg. in weight, were placed in two containers of bicarbonate buffered Ringer solution. One solution contained glucose, 4 mg./ml., and the other did not. During the first sixty minutes of the experiment a slight negative pressure change, indicating that oxygen consumption exceeded carbon dioxide production, was seen. The addition of insulin after sixty minutes to both solutions yielded a final concentration of 10^5 μU./ml. In the solution which contained glucose, a positive gas pressure response was evident within ten minutes and marked by twenty minutes after the addition of insulin. This meant the gas, presumably carbon dioxide, was evolving more rapidly than the oxygen was being consumed, and the respiratory quotient changed from a slight negative to a marked positive value. This evolution of gas was remarkably linear with time and was consistent with the expected increase in production of carbon dioxide (Fig. 1).

In a similar type of experiment, the amount of insulin was lowered and it was found that 1/100 of the initial quantity or 10^3 μU./ml. resulted in a consistent response almost equal (95 per cent) to that shown previously. No exchange of gas occurred in the absence of glucose and as little as 0.125 mg./ml. of glucose resulted in a positive response (slow and not linear with time). In other experiments, in which fat pads were placed in media containing glucose and insulin (added to one of the media), an identical result was obtained, indicating that in the absence of insulin the tissue appears unresponsive to glucose.

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Since this system offered the opportunity to compare possible ultrastructural changes in adipose cells with known metabolic changes in function, sections of incubated adipose tissue were examined under the electron microscope.

In order to be sure we were getting any biochemical effects of insulin, we used the identical procedure to monitor each experiment in which the tissue was examined with the electron microscope. The experimental tissues were removed from the containers ten, twenty, thirty, fifty and sixty minutes after the glucose and insulin were mixed, usually after twenty minutes, since by that time a marked response was evident. Control tissues, incubated in either glucose or insulin but not both, were taken at the end of the equilibration period, and at the same times as the experimental tissues. The small pieces of epididymal fat pad were fixed in cold 1 or 2 per cent osmium tetroxide, dehydrated in increasing concentrations of ethyl alcohol and embedded in butyl methacrylate. Thin sections, 3-400 Å in thickness, were cut with a Porter-Blum microtome and examined under an RCA EMU 2E electron microscope.

Mature yellow fat cells contained a huge droplet of lipid surrounded by a thin rim of cytoplasm. The cytoplasm contained occasional organelles, especially in the region of the nucleus which occurred in the thickest portion of the cytoplasm and bulged into the fat droplet. In the studies with the electron microscope, regions of cytoplasm were selected. Those regions where the cytoplasm occurred as an extremely thin structureless rim (1-2000 Å in diameter) were avoided, and those areas where the thickness of the cytoplasm was at least several times greater than 1-2000 Å and in which cytoplasmic organelles were numerous were studied.

In the specimens not subjected to insulin, the cytoplasm was homogeneous, dense, granular and contained some typical mitochondria. However, it contained only scanty reticulum, and practically no small droplets of lipid. In the experimental tissues subjected to insulin and glucose for a short period of time, a pronounced morphologic change was observed.

The plasma membrane was invaginated at many sites to form minute indentations. Numerous tiny vesicles were arranged in relation to the plasma membrane, suggesting that they might have been formed from a pinching off of a recessed tip of such a fold. Occasionally, deep membranous lined channels, connecting to the surface membrane, were apparent. Deeper in the cytoplasm, especially in the specimens that had been incubated for a longer period of time, numerous large, smooth, membrane-bound vesicles were seen. These vesicles appeared to form a discontinuous system of smooth reticulum from the plasma membrane and its infoldings to the interior of the cytoplasm. In addition, there was a loss of granularity of the cytoplasm and small droplets of lipid were found frequently. Although minute vesicles were seen bordering the plasma membrane in the control specimens (incubated with glucose but not insulin), they were sparse, and no cytoplasmic membranous system of vesicles or channels was found. Tissues, incubated with insulin in the absence of glucose, showed similar morphologic changes to tissues incubated in both solutions.

These morphologic changes, apparently caused by minute quantities of insulin, are reminiscent of the process of pinocytosis, described by W. H. Lewis[6] in 1931, in which the surface of the cell and adjacent cytoplasm are in a state of vigorous activity with an orderly flow of vesicles from the cell membrane to the interior of the cell. This type of membrane activation (flow) may be an important part of a type of active transport mechanism, which carries molecules from the surrounding medium (glucose) into the adipose cells. As such, this hypothesis fits the striking morphologic change which occurs with the addition of insulin to adipose tissue, involving the cell membrane, the formation of surface pinocytotic vesicles and a cytoplasmic membranous reticulum. Glucose is taken into the adipose cells and simultaneously converted into fat.

Whether or not the observed morphologic evidence of pinocytosis is sufficient to account for the rapid rate at which glucose enters the cell, is open to question. It is possible that, as a result of pinocytosis, structural changes may occur in the properties of the plasma membrane of the adipose cell, which result in a more rapid penetration of glucose into the cell. It should be

remembered that the process of vesicle formation results in a loss of surface membrane, requiring the cell to replace the membrane or to make some other adjustment. These studies lend support to the thesis, advocated by Levine et al.[7] and Park[8] that insulin acts by enhancing the entrance of glucose into the cells, and suggest a mechanism by which this may be accomplished.

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DISCUSSION

Dr. Lillian Recant (*St. Louis, Missouri*): Dr. Barrnett, did you have an opportunity to find out whether or not incubated growth hormone or prolactin produced similar changes on your cell membrane?

And secondly, do you have any comment to make about the type of studies Dr. Lacey has done, in which he has studied the effect of glucose infusions on the changes in the beta cells by electron microscopy? The changes, that he describes, in the beta cells look similar to me, because there is an invagination of the membrane and then this formation of droplets. Does this have to do simply with glucose penetrating into a cell, or is this an effect of insulin?

If you incubate fat tissue in glucose alone, little, if any, effect on the CO_2 mechanism is obtained and it is only after the addition of insulin or growth hormone that an acceleration of this process is seen.

Is this really an effect of insulin or could this be simply the phenomenon of glucose penetrating into the cell?

Dr. Barrnett: In answer to your first question, the experiments with prolactin are now in progress, and we are not quite ready to report them.

In answer to your second question, since glucose in the absence of insulin does not cause this morphologic evidence of stimulation at the surface of the cell, we believe, in this particular instance, which is a fairly narrow one, that insulin does stimulate the process of pinocytosis. We also believe, on the basis of the evidence of Holter and his co-workers on amoeba, that a whole hoard of substances will stimulate pinocytosis, and we have found in other instances, examples of this.

I do not think that glucose alone–and I can only say this about adipose tissue–stimulates pinocytosis.

A great many other things have been demonstrated to be carried into the cell by means of pinocytosis. It is difficult for electron microscopists to bring evidence to bear on any arguments that are concerned with soluble and diffusible compounds or ions. However, a large number of small molecules which are electron-opaque or contain electron-opaque atoms have been demonstrated to enter cells exactly by this means.

You may think that since these are solid particles, I am talking about phagocytosis. Where does phagocytosis end and pinocytosis begin? I think we are talking about the same thing.

Dr. Albert Winegrad (*Philadelphia, Pennsylvania*): Dr. Barrnett, first of all, have you found out whether or not some non-specific protein, for example, one of the serum proteins, would reproduce the effect you have described?

And secondly, if you prolong your incubation periods, can you observe pinocytosis?

Adipose tissue taken from normal rats can utilize glucose quite well. If you assume that this is an effect of insulin specifically, might it not just be stimulation of a process which should occur in this same tissue at sometime during a longer incubation period, let's say three hours, because we do know that fair quantities of glucose are taken up, oxidized to CO_2, and converted to fat in this time period.

Dr. Barrnett: Some other non-specific proteins such as protamine or lysozyme are at present being investigated.

In answer to the second point, the longest that we have prolonged our incubation for electron microscope examination was one hour. By this time, the tissue was quite extracted, so we looked no further. The results reported here are based on a comparison of adipose tissue, subjected to both insulin and glucose and of the same tissue of the same animal, subjected to only glucose.

Dr. Doolan (*Kalamazoo, Michigan*): Dr. Barrnett, do you know what happens to the membranes of other insulin-responsive tissues, such as muscle, as well as insulin-non-responsive tissues, presumably such as nervous tissue or, maybe, liver?

Dr. Barrnett: No, I do not. We have done some preliminary experiments on diaphragmatic tissue and liver and obtained some different results.

Dr. H. E. Wertheimer (*Jerusalem, Israel*): Have you pictures of starvation and feeding of carbohydrate?

Dr. Barrnett: Starvation alone causes such morphologic changes in fat that it confuses the picture. We tried this but it is just adding too many variables to the simple system that we have now. So we discarded it after trying two preliminary experiments.

Dr. Jay Tepperman (*Syracuse, New York*): Dr. Oscar Hechter at the Worcester Foundation told us he had been examining some adrenal cortical cells which

had been treated with ACTH, and he felt that the changes he was seeing in the cells of the adrenal cortex under the influence of ACTH stimulation were practically identical with the changes you have been describing.

I wondered whether you have seen his preparations. If so, can you comment on them for us?

DR. BARRNETT: Yes. I look forward to the day when it will be indicated that some hormones act on the cell surface.

DR. RACHMEIL LEVINE (*Chicago, Illinois*): Dr. Barrnett have you considered the suggestion made by Dr. Lazarow when Dr. Ball showed some of these pictures at Woods Hole this summer–the suggestion is in line with some of the earlier observations of Dr. Wertheimer–that is can these vesicles conceivably be glycogen, since the amount of water that had to be taken in would be enormous, and is this really a pocket of water, or is this glycogen prior to the formation of fat?

DR. BARRNETT: I do not believe that it is glycogen which displays a characteristic form in electron microscopic studies of a variety of organs. This form is granular and free in the cytoplasm instead of recurring in vesicles.

In other experiments, using goldthioglucose instead of glucose as our tag, we have found small dense bodies, presumably due to density of gold with the vesicles.

DR. JAMES SALTER (*Ontario, Canada*): What happens if you just add insulin alone?

DR. BARRNETT: The same thing. Insulin without glucose causes the same degree of pinocytosis. The pictures are such that you cannot tell one from the other.

DR. HERBERT S. ANKER (*Chicago, Illinois*): I believe there are values available for the rate of diffusion of water through cell walls. Can you calculate if the water intake is compatible with this exchange?

Furthermore, can you calculate the quantity of protein required in order to make the walls of your vesicles and see if that is compatible with the quantity of protein synthesis going on in these cells?

DR. BARRNETT: First of all, we have no idea, if insulin is stimulating this curious activity of the cell membrane, whether each vesicle that is formed depends upon a molecule of insulin or whether insulin stimulates a unit area of cytoplasm, which reacts in this way.

Secondly, we have no idea of the rate of the number of vesicles that are formed per given area of tissue. Other changes involving mitochondria and ground cytoplasm also occur in addition to pinocytosis, so that the degree of protein synthesis may not be a true measure.

DR. ANKER: Is there any way of spreading out the cell membrane by destroying the volume of the cells, so that you have a two-dimensional picture of the surface only, and then counting the pinholes, so to speak?

DR. BARRNETT: This could not be done easily. The process of pinocytosis, as it occurs here, is one in which there are sites of extreme activity, moderate activity and no activity. To say that everything is going on at a uniform rate would be a mistake.

DR. LEVINE: I know of some experiments of Dr. Bloom at the National Institutes of Health showing that under the influence of insulin, tritium-labeled water does not enter faster into an insulin-sensitive tissue. This is one way of estimating whether the water is sufficient for this kind of process. Whether this is anything definitive or not, I do not know.

DR. BARRNETT: In our experiments, control tissues took up more water than the experimental ones. Remember that this also may be an excretory system as well as an intake system. There is no reason why a vesicle cannot make a roundtrip.

DR. JOHN R. BROBECK (*Philadelphia, Pennsylvania*) How do you know that the insulin is not inhibiting the dissolution of these things rather than stimulating the formation?

DR. BARRNETT: Without insulin, as in the control preparation, vesicles do not occur. You add insulin and they occur.

DR. BROBECK: This could be an inhibition.

DR. BARRNETT: I am assuming that lack of pinocytotic vesicles is an inhibition. Therefore, the presence of them is a stimulation.

DR. TEPPERMAN: I would like to get back to the earlier comment that was made about the possibility of all hormones eventually being shown to act by virtue of their effects on cell surfaces. We find that there are tremendous difficulties inherent in taking this position.

We have in mind the experiments of Randle, which are now becoming quite well known, in which the action of insulin is made to be analogous to certain effects of inhibitors, in anaerobiosis, or on the permeability of cells to glucose, for example. In the presence of anoxia or hypoxia, which are inhibitors of metabolism, glucose enters the cell more readily than it does in their absence.

Dr. Randle has made the intriguing suggestion that there is a "keeper-out-ase," if you will, of glucose, which is powered by some type of metabolic machinery in the cell, and that insulin may exert its effect by inhibiting or unccupling the power from the "keeper-out-ase," thus permitting the glucose to get into the cell.

If there is any merit at all in this suggestion then there are all sorts of possibilities for hormones working on permeability mechanisms by actually exerting their effects on the machinery inside the cell that is coupled with the permeability mechanism.

It seems possible to me that in this particular tissue there may be deep kinds of effects of insulin inside the cell which would then express themselves in some of the changes which you may have seen. I am especially intrigued by the fact that you find these changes when you do not have glucose coming into the cell. The insulin works in the absence of glucose.

DR. BARRNETT: I do not see how the work of Dr. Randle bears on the present problem with which we are faced. Pinocytosis will probably end up being

stimulated by a great many things. One of the questions that is of import is the specificity of pinocytosis.

I am not surprised that anoxia will increase the degree in which materials get into the cell. Anoxia, for example, will increase the degree in which materials will traverse capillary beds.

On the one hand, we know that glucose is not getting into the cell without insulin and, on the other hand, we know that glucose is getting in with insulin. I believe, that there is a morphologic basis of function: and when I see this difference between the presence and absence of membranous activity of the plasma membrane, I am forced to attribute this thing to the change in function that has been observed.

DR. LEVINE: Dr. Barrnett, couldn't you resolve this problem of the functional connection of this morphologic change with the actual effect of insulin by doing exactly the same thing in a tissue the carbohydrate metabolism of which is not stimulated by insulin?

If pinocytosis does not occur then the insulin function and pinocytosis have something in common.

If pinocytosis still occurs, then I would be hard pressed to put the two things together.

The other thing, which I brought up when Dr. Ball presented some of the data, was to explain the specificity by which optical rotation can be linked with such an issue as pinocytosis.

DR. WINEGRAD: If we accept the work of Dr. Barrnett on the basis of his own interpretation, then I think we must make the assumption that the cell is perfectly capable of taking up glucose and oxidizing it to CO_2 in the absence of demonstrable pinocytosis, because in the particular preparation which he used, when the cell is incubated with glucose, there is no difficulty in demonstrating glucose utilization.

I would like to know how he resolves this conflict.

DR. BARRNETT: I did not realize the conflict existed. Maybe Dr. Renold could help me out on this point.

DR. ALBERT E. RENOLD (*Boston, Massachusetts*): It is just a matter of rate. It is true, speaking strictly, glucose does not get in unless there is pinocytosis. Yet there is difficulty associating that with the fact that if there is CO_2 production, glucose is getting in. But there may be a basal rate at which it can get through as well as a much faster rate following specific stimulation.

DR. J. A. F. STEVENSON (*Ontario, Canada*): Since you prefer the term pinocytosis to phagocytosis, it would seem to me that this implies that this mechanism is more concerned with the solvent than the solute. It might then follow that you think this is an important factor in the movement of water from one direction across the membrane to another.

Do you think that this process is involved in the movement of water, and have you looked at cells under conditions of acute hydration and dehydration?

DR. BARRNETT: I have not. My hunch would be that as the fluid medium, bathing the cell, enters it, pinocytosis may occur.

DR. F. X. HAUSBERGER (*Philadelphia, Pennsylvania*): If you incubate adipose tissue in one of the usual buffers, even with the addition of albumin, then the adipose tissue takes up water to the extent of about ten times the amount which is normally present in the adipose tissue. How does it get in without insulin?

DR. BARRNETT: I will just repeat the point that I made: that, as in the amoeba, maybe a whole hoard of substances will stimulate pinocytosis, in adipose tissue and in other cells.

DR. F. X. HAUSBERGER: If you incubate adipose tissue with glucose in a relatively low concentration, let us say 100 mg. per cent, and incubate another sample in 400 mg. per cent, there is a significant rise in CO_2 production and lipogenesis, due to the increase in the glucose concentration.

The increase is not so dramatic as if insulin was administered. Insulin increases it, maybe, six, eight, ten or twelve times. But still the increase may be twofold. Would there be any increased pinocytosis in this case?

DR. BARRNETT: I would say yes to this, just as a guess.

The Etiologic Mechanism of Some Forms of Hormonally Induced Obesity

F. X. HAUSBERGER, M.D.* AND B. C. HAUSBERGER†

ENDOCRINE FORMS of obesity, with the exception of those associated with diseases of the adrenal cortex, are generally considered to be rare. This presentation attempts to demonstrate that certain forms of spontaneously occurring adiposity in mice, as well as adiposity occurring after castration, are probably caused by hyperfunction of the adrenal cortex and by increased secretion of insulin. The latter is suggested by the hypertrophy and hyperplasia of the beta cells of the pancreas.

EXPERIMENTAL HYPERADRENOCORTICISM

Insulin accelerates fat deposition by, and growth of, adipose tissue and produces this effect also in the presence of large amounts of cortisone. Rats treated with cortisone develop infections frequently and lose weight. Simultaneous administration of insulin promotes, even in animals with infection, increased storage of fat and growth of adipose tissue, without preventing loss of body protein.[1,2] Rats receiving cortisone show minimal or no islet hypertrophy. On the other hand, enlargement of the islets in Cushing's syndrome has been described by several investigators.[3–5] Islet enlargement was found subsequently in other animals with hyperadrenocorticism.[6,7] Recently, we noticed it in mice with ACTH-secreting anterior pituitary tumors.[8,9] This tumor and its effect on the host, the LAF_1 mouse, have been extensively described by Furth and his associates. There is ample evidence that the tumor secretes only ACTH, and that it produces the typical symptoms seen in Cushing's syndrome, especially obesity.[10–13] The islet hypertrophy, which often is remarkably extensive, always parallels the degree of the obesity (Figs. 1 and 2). Adrenalectomy prevents both islet hypertrophy and adiposity but not growth of the tumor. The consumption of food by "tumor mice" during periods of rapid weight gain is increased. Reduction of the food intake to an amount sufficient either to maintain normal body weight or to induce slight weight loss, does not prevent islet hypertrophy. The enlargement of the islets in these animals is distinctly present although it is less than that in tumor mice given food *ad libitum*.[9]

It is interesting to note that in tumor mice maintained on limited food intake considerably more fat was deposited than in the control mice (Fig. 3).

Kendall[14] has demonstrated that implanting of pellets of compound A (dehydrocorticosterone) or B (corticosterone) subcutaneously induces adiposity in mice. It should be pointed out, however, that only certain strains respond in this manner. Repeating Kendall's experiments, using LAF_1 mice, islet hypertrophy, similar to that observed in obese tumor mice, was found in all animals which became markedly obese (Fig. 4).[15] Administration of compound A or B to mildly alloxan diabetic mice of the LAF_1 strain caused, as is to be expected, exacerbation of the diabetic condition, loss of weight and fat and ultimately death.

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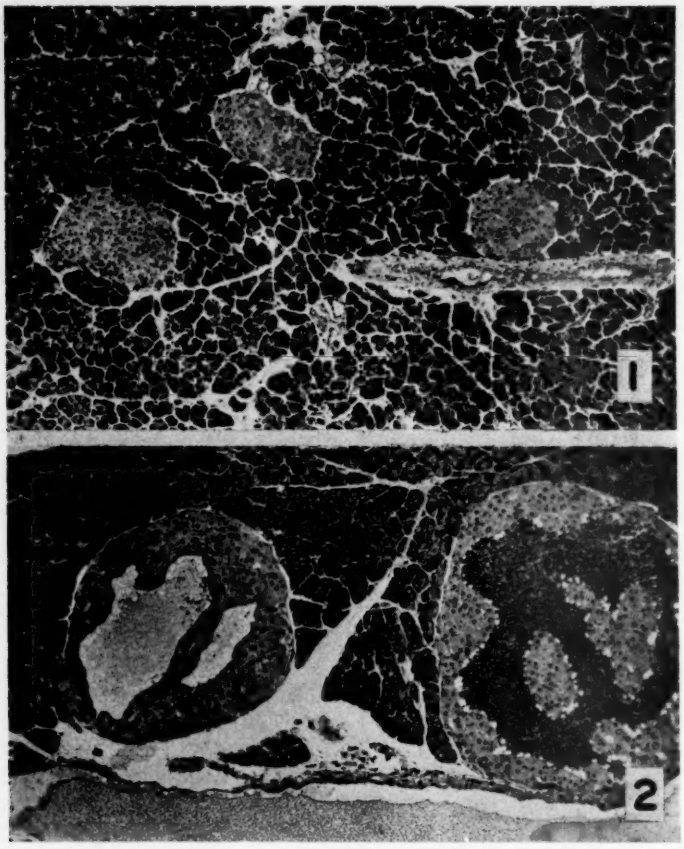

FIG. 1. Photomicrograph of the pancreas of a LAF_1 mouse, weighing 24 gm The islets are of normal size, the beta cells contain large amounts of beta granules. Original magnification ×100.

FIG. 2. Pancreas of a tumor mouse, weighing 42 gm. The islets are greatly enlarged and contain predominantly hypertrophic and hyperplastic beta cells. Most of these cells show complete or partial loss of their granules and an activation of the Golgi apparatus. These signs are considered to be indicative of enhanced release of insulin. Cavitation of the islets is common in most tumor mice weighing more than 40 gm. Original magnification ×100.

In rats in which obesity is induced by hypothalamic lesions or by force feeding minimal or no islet hypertrophy develops.[15] Furthermore, in hyperphagic rats with induced hypothalamic lesions which were maintained for six weeks on the preoperative weight, fat and protein were present in the same amounts as in intact control animals.[16]

Hypertrophy and hyperplasia of the beta cells, loss of beta granulation and activation of the Golgi apparatus in mice with hyperadrenocorticism suggest an increase in the secretion of insulin, even though no direct proof has been found as yet. Several other observations, too numerous to be mentioned here, support this theory.

HEREDITARY OBESITY

Spontaneously occurring obesity has been described in a few strains of mice. The adiposity in hereditarily-hyperglycemic mice (from the Roscoe B. Jackson Memorial Laboratory)

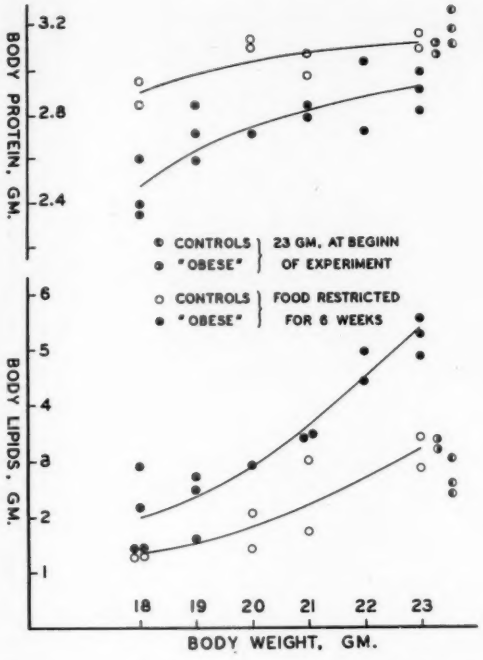

FIG. 3. Body weight, fat and protein in LAF_1 control mice and of tumor mice with potential obesity receiving limited food. The tumor was grafted into a large number of mice. Four weeks later, before the tumor started to grow and to secrete ACTH (judged by the weight of the adrenals), one group of control mice and one group of tumor mice were killed. At this time, the protein content was the same in the animals in both groups; the fat content of the tumor mice was insignificantly higher. Other groups of control mice and tumor mice of the same body weight (23 ± 0.5 gm.) were kept for an additional six weeks and maintained on restricted amounts of food. The tumor mice gained fat or lost considerably less fat than the control animals despite loss of weight and increased loss of body protein.[9,14]

is most likely due to hyperinsulinism, according to Mayer and co-workers,[17,18] but there are no observations indicating that hyperadrenocorticism is a causative factor. Adrenalectomy with subsequent maintenance of DOCA® does not prevent obesity,[19] nor does this procedure induce weight loss in animals which are already obese.[20]

The adiposity in NH mice, in yellow obese mice, and possibly in the New Zealand strain of mice seems to be caused by some form of hyperadrenocorticism. In NH mice, obesity is quite regularly observed when the animals are a year old. Early castration reduces this time to about six months. Excess weight gain in normal and castrated animals is always associated with subcapsular hypertrophy of the adrenal cortex and frequently with cortical adenomas[21,22]; in female mice which have not yet been castrated, early disappearance of the ovarian follicles has been noted.[23] Adrenalectomy with maintenance on DOCA induces weight loss in castrated and non-castrated obese NH mice, but not in mice with obesity induced by the administration of aurothioglucose.[24]

We investigated normal and castrated NH mice at all weight levels. Enhanced weight gain was always found to be associated not only with cortical hypertrophy but also with enlargement of the islets.[25] Adrenalectomy before the onset of obesity prevented both excess weight gain and islet hypertrophy.[20]

In the yellow obese mouse, the maximal degree of obesity is reached during the ages of seven to eighteen months; female mice are usually heavier than males. Yellow female mice cease to reproduce earlier than their non-yellow litter mates. Ovarian anomalies and failure to obtain typical estrus smears are common.[26] Normal litter mates of yellow obese mice after castration tend to become as obese as many non-castrated yellow mice.[27,28] Silberberg and Silberberg[29] found islet hypertrophy in obese yellow mice and regressive changes in the neurons of the thalamus, hypothalamus and cerebellum. They considered the possibility that hypothalamic dysfunction is the cause of obesity.

We compared the pancreas and the adrenals of yellow mice at all ages and weight levels and confirmed the enlargement of the islets in obese yellow mice. In addition, we always found in yellow obese mice a considerable subcapsular hypertrophy, frequently of the nodular type, comparable to that observed in NH mice.[25] Subcortical hypertrophy is also rather frequently seen in normal siblings of yellow obese mice, especially the older ones. However, the degree of this hypertrophy is much less pro-

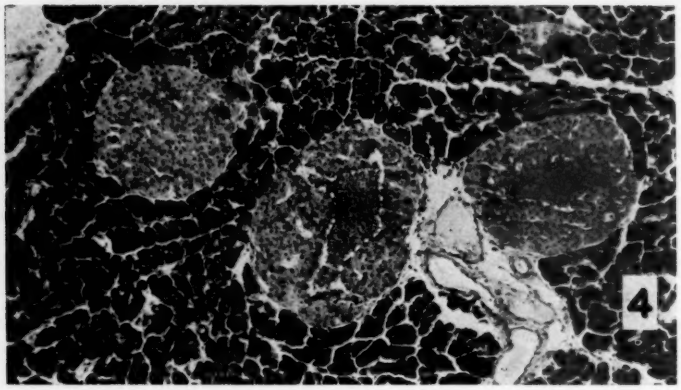

FIG. 4. Pancreas of a female mouse implanted with pellets of compound B for five months. The islets are greatly enlarged. The body weight of this animal was 37 gm. and its fat content 16 gm. The weight of the control animals ranged from 24 to 27 gm. with a maximum fat content of 3.9 gm. Original magnification ×100.

nounced than in the obese yellow mice (Figs. 5 and 6).

In order to clarify the possible etiologic role of the adrenals, obese yellow mice were adrenalectomized and maintained on DOCA. In contrast to adrenalectomized hypothalamic hyperphagic rats and mice, the yellow mice lost their excess weight and did not regain it. Adrenalectomy prior to the onset of obesity also prevented islet hypertrophy.[20] Young yellow mice joined by paraboitic union to normal litter mates fail to become obese, according to Weitze,[30] again in contrast to hypothalamic hyperphagic rats. Extending Weitze's experiment, we found that paraboitic union between yellow and non-yellow twins prevented, in addition, the marked hypertrophy of the adrenal cortex and the enlargement of the islets in the yellow partner.[20]

A fourth form of hereditary adiposity has recently been described by Bielschowsky and Bielschowsky[31] in the New Zealand strain of mice. Among the salient changes are again enlargement of the islets of the pancreas and "slight nodular hypertrophy of the adrenal cortex." The blood glucose levels are elevated and the water consumption is increased. Pregnancy as well as the administration of stilbestrol lower the blood glucose levels and delay the onset of obesity.

CASTRATION

The functional status of the gonads has long been recognized as having an influence on the secretion of the adrenal cortex. Castration is frequently followed by compensatory hypertrophy of the adrenal cortex but not necessarily by an increase in the weight of the adrenals.

Sometimes, castration, or changes in gonadal secretion are followed by accelerated gain of body weight, depending on age, sex, species and strain. The onset of obesity in NH and yellow mice, and probably in the New Zealand strain, seems to be associated with changes in the activity of the sex glands. This possible association motivated us to investigate the effect of castration on the relation between increase of body fat, hypertrophy of the adrenal cortex and the islets in several strains of mice, in guinea pigs and in hamsters.[25]

The response to castration in mice depends on the strain. The fat content of LAF_1 mice ten months after castration is approximately twice as high as that of the control animals; the former show considerable subcapsular hypertrophy of the adrenal cortex and enlargement of the islets. In CE mice, gonadectomy produces consistently enhanced storage of fat and hypertrophy of islets and adrenal cortex.

Castrated Syrian hamsters always deposit

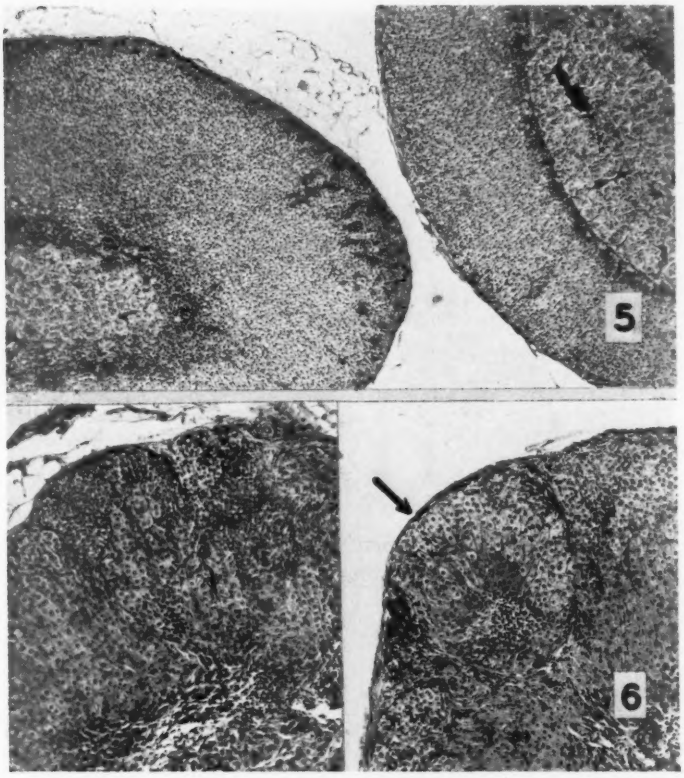

FIG. 5. Adrenal of a yellow obese mouse (*left*) and of a normal sibling (*right*), both seven months old and weighing 48 gm. and 24 gm., respectively. The adrenal of the yellow mouse shows dark bands of dense spindle-shaped cells, extending from the glomerulosa into the fasciculata. Original magnification ×50.

FIG. 6. The two photomicrographs demonstrate more extensive subcapsular hypertrophy in obese yellows. In some areas the regular arrangement of the cortical cells is greatly disturbed; cells often form adenoma-like clusters, sometimes adenomas (arrow). Original magnification ×100.

larger amounts of fat than do the control animals, and the former are frequently larger in size (Fig. 7). Adrenals of hamsters (at least in the age groups used in our experiments) show no spontaneous hypertrophy of the cortex (Fig. 8). Therefore, the marked subcapsular hypertrophy after gonadectomy (Fig. 9), as well as the enlargement of the islets of the pancreas (Figs. 10 and 11), can readily be recognized.

In guinea pigs, marked islet hypertrophy develops soon after castration. The changes in the adrenal cortex and islet system, the fat deposition and the growth rate occur in these animals apparently in cyclic form. The morphologic changes in the adrenals and the pancreas again are associated with excess fat deposition.

It should be noted that hyperadrenocorticism as well as castration are associated with increased fat storage only in certain species and strains, sometimes only in certain subjects. In male human beings castrated during childhood, Tandler[32] described two types, the fat

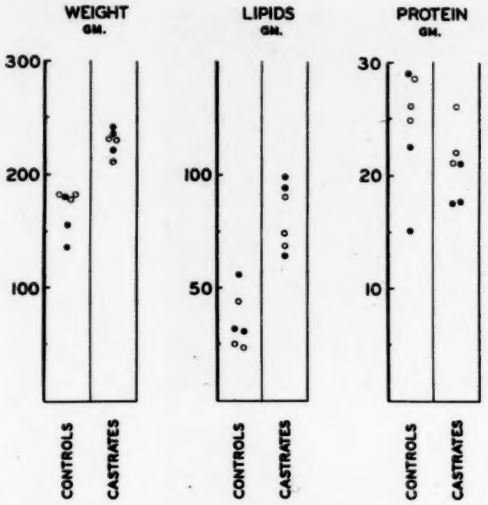

FIG. 7. Body weight, fat and protein content of normal hamsters, and of litter mates which were castrated at the age of twenty-one days; both groups were killed at the age of twenty-one weeks. The fat content of the castrated hamsters is twice as high as that of the control subjects. ● = Males, ○ = Females.

and the tall. Several other reports, especially in the older literature, indicate that the islet system and the adrenals are involved in human obesity. Ogilvie[33] and Kup[34] noted islet hypertrophy in more than half of their obese patients.

Simpson[35] found in a certain form of obesity in children an increased urinary excretion of hydrocortisone. Increased excretion and turnover of hydrocortisone in obese persons was also noted by other investigators.[36] De Salcedo[37] and Zeyneck[38] reported a high incidence of adrenocortical hyperplasia in overweight patients. As outlined in this presentation, the frequent involvement of the adrenal cortex and islet system in obesity of human beings and animals suggests a causative relationship, although direct proof is missing.

SUMMARY

Hyperadrenocorticism causes obesity and enlargement of the islets of the pancreas due to hypertrophy and hyperplasia of the beta cells, suggesting accelerated release of insulin. It seems unlikely that an excess of circulating corticosteroids can produce obesity directly, since administration of corticosteroids which induces adiposity in control animals, fails to do so in mildly alloxan diabetic animals. Considerable reduction of the food intake does not prevent the increased fat deposition and islet hypertrophy in hyperadrenocorticism. Some strains of mice develop adiposity spontaneously and simultaneously show subcapsular adrenocortical hypertrophy and enlargement of the islets. All these changes can be prevented by adrenalectomy. Adrenalectomy, however, does not affect development of hypothalamic obesity. The obesity seen after castration in animals of suitable species and strains is also associated with hypertrophy of the adrenal cortex and the islets of the pancreas.

It is thought that reduction or cessation of the activity of the gonads induces changes in

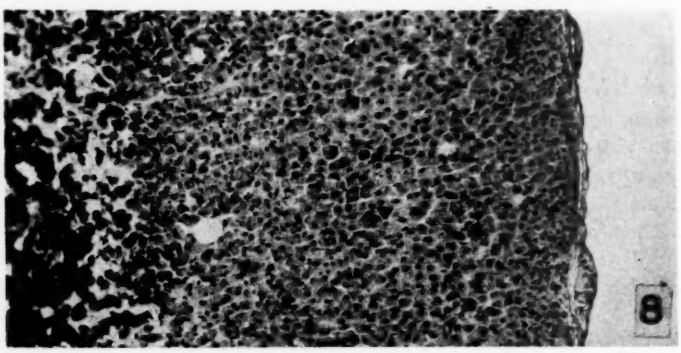

FIG. 8. Adrenal of a control hamster, demonstrating the normal histologic appearance of the cortex. Original magnification ×100.

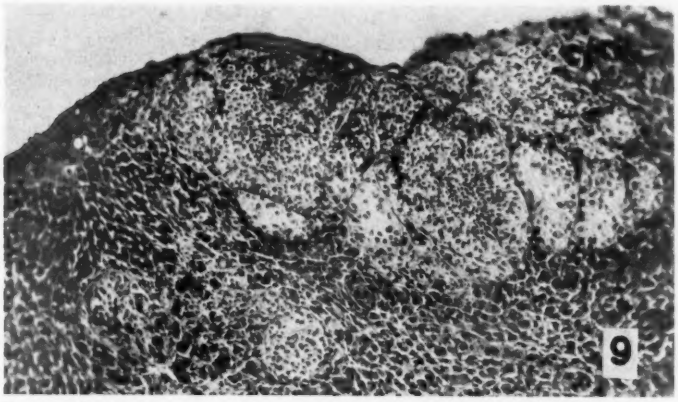

FIG. 9. Extensive subcapsular hypertrophy, typical for castrated hamsters Several investigators have* described almost identical changes in mice after castration. Original magnification ×100.

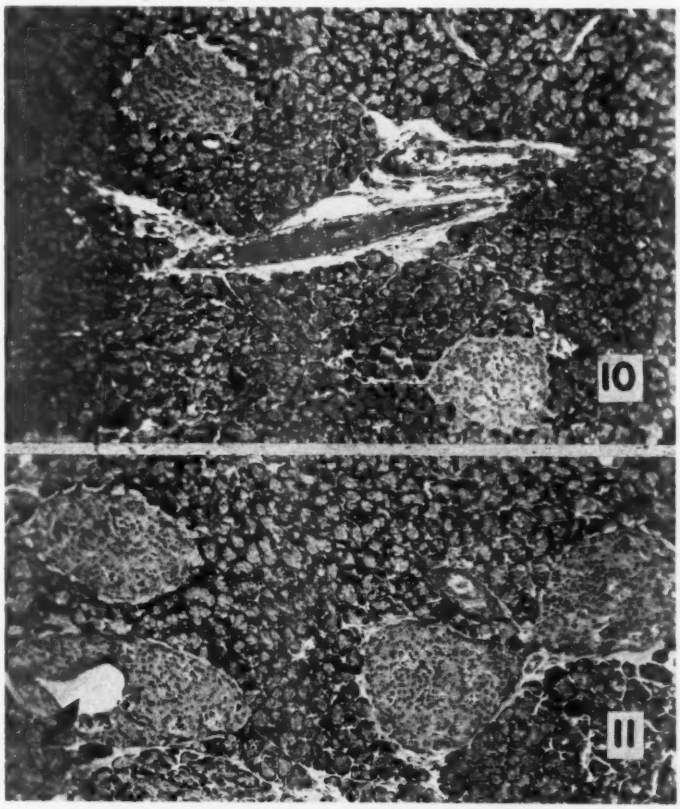

FIG. 10. Pancreas of a control hamster with islets of normal size and appearance. Original magnification ×100.

FIG. 11. Pancreas of a castrated hamster with a greater number of larger islets due to hypertrophy and hyperplasia of the beta cells. One islet (arrow) shows cavitation. Original magnification ×100.

* For references see Gardner, W. U. *Advanc. Cancer Res.*, 1: 198 1953.

the secretion of the adrenal cortex. This in turn stimulates increased secretion of insulin which produces adiposity.

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DISCUSSION

Dr. F. D. W. Lukens (*Philadelphia, Pennsylvania*): There is a great deal of conversation among investigators as to the meaning of certain animal experiments in man. Now, it may be that the species of animal used by Dr. Hausberger are easily capable of islet hypertrophy, and the use of these species, therefore, would suggest that man may be subjected to similar stimuli, even if the response of the islets in man is quite different.

Of course, there are peculiar exceptions. I happen to think of them mostly from clinical experience. What about the patient with an islet cell adenoma who does not become obese? There are certainly such patients on record.

That leads to the question that the endocrine glands will not do 100 per cent of this without good dietary intake, that is, increased food intake. Have you ever given adrenocortical hormones or transplanted adrenal tumors, if that is what they were?

Dr. F. X. Hausberger: They were ACTH-producing tumors.

Dr. Lukens: Have you ever done this and then kept the animals in a constant diet, the same as the control group?

Dr. F. X. Hausberger: Yes. I cannot recall the exact amount but it definitely was subnormal. However, islet hypertrophy did develop.

Dr. Lukens: It was a paired feeding experiment, and still island hypertrophy developed?

Dr. F. X. Hausberger: Yes, and the blood sugar was lower than normal.

Dr. Lukens: Did these animals then become obese?

Dr. F. X. Hausberger: They became relatively obese. The control animals had about 3 gm. of fat, the others 6 gm.

Dr. Jay Tepperman (*Syracuse, New York*): Were the total weights the same?

Dr. F. X. Hausberger: Yes the weights of the animals were the same. They were all kept on a 23 gm. level.

Dr. Lukens: Dr. Tepperman rightly reminds both of us that when an animal with excessive adrenocortical hormone becomes obese, it is also losing a little protein.

Dr. F. X. Hausberger: Yes, that was also shown here.

Dr. Ramey (*Washington, D. C.*): I am a little confused in one regard. What about the connection between obesity and diabetes, with a diminution, in many instances, of production of insulin, together with the size of the islet cell? This would be almost the opposite situation to what you have described, particularly in man.

Dr. F. X. Hausberger: You mean whether such animals have diabetes?

Dr. Ramey: No. In man the connection has been made so frequently between diabetes and obesity.

Dr. F. X. Hausberger: That is a question I can only speculate on, as is true probably of everyone else.

Dr. Lukens: There is a little bit more than speculation, if I may intervene in this discussion. It is not as accurate as we would like to have it. But Dr. Ogilvie, pathologist of Edinburgh, has observed a moderate degree of islet hyperplasia in obese human beings. Dr. Jerome Conn and his associates have recorded somewhat overactive glucose tolerance curves —they tend to react with a low level of blood sugar at the end of the curve—years before diabetes develops, in certain people.

So, there is some evidence that a process like this, occurring in a species which cannot respond with as much islet hyperplasia, and occurring, perhaps, for years instead of for a mere matter of weeks or months, might make just the difference which you ask about.

Dr. Hervey (*Sheffield, England*): I was very interested in the parabiotic experiment, Dr. Hausberger.

Am I right in thinking that you found that parabiosis with a normal mouse would prevent the development of obesity in the hereditary strain in which it would otherwise have developed? Do you have any suggestions as to the explanation of that? Would you elaborate on that experiment, because I am not quite clear what happened.

Dr. F. X. Hausberger: It was shown, I think, twenty years ago, that if one parabioses a young, potentially obese, yellow mouse, before it becomes visibly obese, with a normal litter mate (this obesity is a dominant type of obesity, connected with a yellow skin color) obesity does not develop in the potentially obese mouse. If you separate them, then it develops.

Since we thought that the obesity in the yellow mouse might be related to hyperadrenocorticalism and subsequent island hypertrophy, we were interested in seeing whether the development of the morphologic changes

in the adrenals and the pancreas would be prevented. When we paraboised a yellow obese mouse and a normal sibling, the obesity was prevented and the morphologic changes in the adrenals and the pancreas were prevented. Adrenalectomy has the same effect in the potentially obese mouse.

DR. C. N. H. LONG (*New Haven, Connecticut*): Dr. Hausberger, I would like to ask for a little clarification. Is the thesis here, in the development of obesity in certain human subjects, that the overactivity of the adrenal comes first, and then this is followed by the excessive deposition of fat?

DR. HAUSBERGER: I don't know.

DR. LONG: But I would point out without going to the question of human obesity, Drs. Brobeck and Tepperman showed some time ago that by carrying out a partial pancreatectomy on rats before one induces the hypothalamic lesion, on a given intake of food, there was no glycosuria or hyperglycemia; but merely the presence of the hyperphagia would progressively increase the severity of the diabetes. Finally, when you brought the rat back to the initial food intake, there was then a marked degree of glycosuria.

Under those circumstances, it is quite obvious that it was the excess feeding of the food, in the presence of a pancreas that was made deficient, that produced the diabetes.

I wondered whether your view was that it might be the other way around: that you could have the overactivity of the adrenal cortex breaking the pancreas, rather than excessive food intake.

DR. F X. HAUSBERGER: There is one clinical investigation by Cohen, in *The British Medical Journal*, in which this investigator showed that obese children excrete more corticosteroids than normal ones. However, when he reduced the food intake drastically to a starvation diet, the excretion went back to normal. Since he did no control experiments to demonstrate how much it would be reduced by the same food regimen in normal children, it is very difficult to say what was first and what was second.

DR. LUKENS: I think we might well agree that what Dr. Long and Dr. Hausberger have brought out is that the role of food intake or spontaneous development of the adrenal cortex is something that cannot be clearly distinguished, certainly not in man.

DR. JAMES M. SALTER (*Toronto, Ontario, Canada*): Dr. Hausberger, did you say that the animals with the hyperadrenocorticalism had the same food intake and yet were obese when compared to their controls?

DR. F. X. HAUSBERGER: Yes. Even with a smalier food intake than the controls, they deposited more fat.

DR. SALTER: Were they the same weight?

DR. F. X. HAUSBERGER: They were the same weight, yes. Even with a partly lower weight, they still had a higher fat content.

DR. SALTER: If they were the same weight, how can that be?

DR. F. X. HAUSBERGER: They lost protein.

DR. SALTER: The caloric values of the animals would still be quite different. If they are the same weight, and one contains more fat than the other even though it has got less protein, the caloric value of the animal with the fat will be much higher.

DR. F. X. HAUSBERGER: Maybe their activity was reduced. That is quite possible.

DR. SALTER: That is what I wondered. Was there some change in their activity or the metabolic rate or something?

DR. F. X. HAUSBERGER: The expenditure had to be lower in these animals. Otherwise, they could not have saved some calories in order to deposit these calories in the form of fat.

DR. JEAN MAYER (*Boston, Massachusetts*): I would like first to confirm what Dr. Hausberger said about those ACTH-tumor mice, and extend the discussion to this extent: I think one of the confusing ideas that we must eliminate is to think of obesity as a syndrome and to always try to get general conclusions on the subject.

Obesity is the common end product of a great many conditions which have this in common: there is a positive energy balance. I think that if we try to generalize our thinking about obesity and always want to get similar characteristics, we will be as confused as if we thought about fever as one syndrome.

We note, quite reproducibly, that those types of obesity which depend upon metabolic errors or hormonal errors, of which we have studied a number, have this in common: when you reduce the animals to the same body weight as their controls, the composition still is obese in terms of increased fat content.

By contrast, those obesities which do not depend on a metabolic error, such as hypothalamic obesity or obesity due to conditioning and so on, have this in common: when you bring the body weight back to the control body weight, the body composition is almost the same as normal, with some differences, which I think Dr. Cohn may talk about, which probably are dependent upon the manner in which the animal eats.

DR. G. C. KENNEDY (*Cambridge, England*): Dr. Hausberger, have you tried, in your non-obese animals, to produce obesity simply by the injection of cortisone? Have you found any level of dosage of cortisone which will increase the appetite?

DR. F. X. HAUSBERGER: Cortisone is not effective, but compound B or A is.

DR. KENNEDY: Earlier, I understand, in the special strains of mice it was used successfully?

DR. F. X. HAUSBERGER: We tried to do it with cortisone. We injected it. Perhaps we should have administered it in very small amounts in the form of pellets. We intend to repeat that experiment in suitable strains of mice. But already, I think, Kendall has demonstrated that cortisone is not effective. As to hypocortisone in mice, I do not know.

DR. KENNEDY: Would you agree that on the whole, particularly if one is unwise enough to raise the dose of cortisone, the effect of cortisone in the intact animal

is usually the reverse: to decrease appetite considerably, rather than to produce obesity?

DR. F. X. HAUSBERGER: Yes. If you administer large amounts of cortisone to mice, they usually become ill from infections and rapidly lose weight if this procedure is continued.

DR. KENNEDY: Yes. But before they lose weight?

DR. F. X. HAUSBERGER: Before they lose weight they show some signs of islet hypertrophy. The changes in the fat content are so minimal, however, that one cannot say whether there was really the beginning of increased fat deposition and then the infection was superimposed or a negative effect of cortisone. I cannot tell. There is some islet hypertrophy.

DR. KENNEDY: There is, obviously, some difference of opinion here. I would have said that the loss of weight antedates the incidence of infection and that many animals lose a great deal of weight under treatment with cortisone and infection never develops. Nevertheless, as you say, they seem to have hyperinsulinism.

DR. WILLIAM PARSON (*Charlottesville, Virginia*): One word about repeating Kendall's studies. We had difficulty with this until we found that using older mice, using DBA mice, compound A pellets would produce the obesity; whereas they would not in the younger mice.

The results of our studies and those of Dr. Mayer, using mice which were given the compound A pellets and then starved, compared with control mice of comparable weights which had been starved, congenitally obese mice (which had been starved), and also with animals who had hypothalamic damage by gold (who had been starved), confirm those of Dr. Hausberger. The groups who had the so-called metabolic obesity, (the compound A and the congenitally obese ones), had a higher fat and a lower nitrogen content of the carcass than did the normal control animals and those animals with hypothalamic damage.

In our studies, using *in vitro* technics with C_{14} acetate, we found that the rate of synthesis of fat was increased in the mice given compound A pellets and the congenitally obese mice but not in C^{14} mice with damage to the hypothalamus.

This all fits very well with Dr. Hausburger's thesis.

DR. LONG: I would like to point out, first of all, that there is a great deal of difference between man and the rat and mouse, regarding the kind of steroids that are secreted from the adrenal glands. It may well be that in the mouse and the rat, in which cortisone and dehydrocortisone are the predominant steroids, these effects can be obtained. But this is not the case in man, in whom the predominant steroids are cortisone and hydrocortisone.

DR. LUKENS: There is no doubt that cortisone and hydrocortisone can induce obesity in man, under many conditions.

DR. F. X. HAUSBERGER: You have shown that hyperadrenocorticism in man also produces islet hypertrophy, at least in Cushing's syndrome.

DR. LUKENS: Yes. Hypertrophy is not seen as vividly under the microscope as it is in your mice

Effects on Metabolism Produced by the Rate of Ingestion of the Diet

"Meal Eating" Versus "Nibbling"

CLARENCE COHN, M.D.* AND DOROTHY JOSEPH†

THE METABOLIC ACTIVITIES of cells appear to be governed by at least three different types of regulating influences. Unicellular organisms serve to demonstrate the point that the genetic background of the cell plays a dominant role in determining its enzymatic activities; the "one gene–one enzyme hypothesis" states that every enzyme present in a cell has been genetically transferred from parent to offspring cell.[1] Secondly, on the basis of increased or decreased availability of substrates, some enzymatic systems may be affected with respect to the quantities of enzymes necessary for the accomplishment of a given physiologic reaction.[2] Finally, in higher organisms, additional modifying influences in the form of hormones have been superimposed on the two factors just mentioned.

On the basis of studies performed in this laboratory over the past five years, it has become apparent to us that the rate of ingestion of the foodstuffs plays a significant role in the regulation of intermediary metabolism.[3–7] It appears that the traffic over multiple alternate enzymatic pathways can be altered by the manner in which food is eaten, i.e, differences in metabolism may be seen, depending on whether calories are ingested in small frequent feedings (hereafter called "nibbling") contrasted with large influxes of food as spaced full meals, (called "meal eating"). The manner of eating appears to change specific, hence ultimately total, metabolic reactions. The data which led to the development of the concept and the implications thereof which are applicable to man are herein summarized.

Respiratory quotient (R.Q.) studies, performed on rats trained to eat their entire daily ration in a limited period of time, provide the first data which can be used to demonstrate that meal eating alters metabolic reactions.[8–10] Experiments, with both the intact animal and with the liver slices *in vitro* yielded results which were interpreted as indicating that increased lipogenesis accompanies the meal eating habit, as compared to the nibbling habit. It may be deduced from Levin's data on force-fed rats[11] that spaced meals cause an alternation in body composition in that body lipids are increased. We now regard force-feeding as essentially a variant of meal eating.

Our attention was directed to the effects of force-feeding on body composition in studies which were designed to determine the reason for the leanness of the adrenalectomized animal.[3] In order to evaluate the effects of food intake on the reported results, adrenalectomized rats, both those being force-fed and those having free access to food were studied. Normal control rats which were similarly fed

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TABLE I
Changes in Body Fat of Rats Fed *Ad Libitum* or Force-Fed for Two Weeks*

Body Composition	Manner of Feeding	
	Ad Libitum (8)†	Force-Fed (8)†
Original		
Weight (gm.)	148	159
Fat (gm. per rat)	20.1	15.3
Fat (% body weight)	13.5	9.6
Final		
Weight (gm.)	212	222
Fat (gm. per rat)	20.4	39.0
Fat (% body weight)	9.6	17.6
Changes		
Weight (gm.)	64	63
Fat (gm. per rat)	0.3	23.7

* Data from Cohn et al.[3]

† The number of animals in the group. The values given are the mean values of the group. The original composition is based on analysis of rats from the same groups killed at the beginning of the experiment.

were observed also. The force-fed animals were given twice daily an amount of food by stomach tube that would "pair gain" them against those eating *ad libitum*. After three weeks, it was found that the normal force-fed rats contained almost double the amount of body fat as did their nibbling counterparts which had access to food twenty-four hours per day (Table I). Further studies have demonstrated that the difference in body fat was not related to the presence or absence of the adrenals, the sex of the animals, the protein in the diet, the handling of the animals, the dilatation of the stomach that accompanies force feeding, or to the administration of the food during the day or night.[4,12] No differences in the fecal fat excretions of either group were found which might have accounted for the differences in body composition, nor were differences in body activity seemingly responsible for our findings.

Since all our studies had been performed under the conditions of pair gaining, the possibility existed that the force-fed animals had in all actuality received, and hence retained, more calories than those which ate *ad libitum*. In order to obviate any possibility of such a discrepancy, the experiments were repeated,

TABLE II
Body Constituents Gained by Rats Eating *Ad Libitum* and Those Pair Fed Force-Fed Against Them*

Weight Gain	Manner of Feeding	
	Ad Libitum	Force-Fed
Body weight gain (gm.)	58	57
Percentage of weight gain, attributable to:		
Water	67.5	54.8
Fat	7.7	23.2
Protein	22.3	17.4

* Data from Cohn and Joseph.[5]

"pair feeding" the force-fed animals against those with free access to food, and furthermore, the animals were fractionated into fat, protein and water at the time they were sacrificed. The meal eating animals contained more body fat and less protein and water than did the nibbling rats against which the former were pair fed (Fig. 1 and Table II).

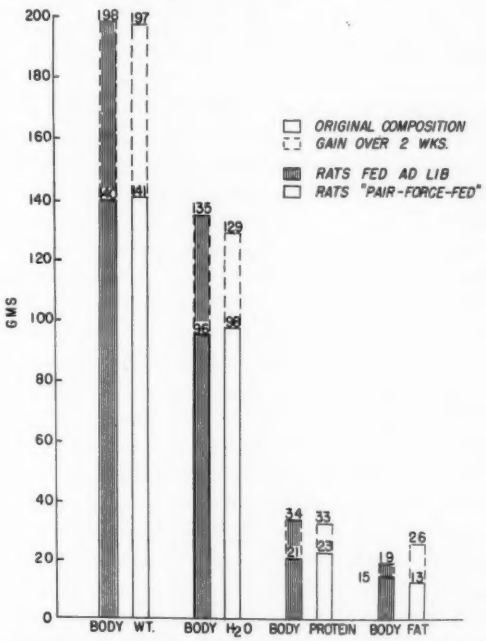

FIG. 1. Changes in body weight and body constituents of nibbling and force-fed rats pair fed against each other. The original body composition was derived from control animals in each group which were killed at the start of the feeding period.[5]

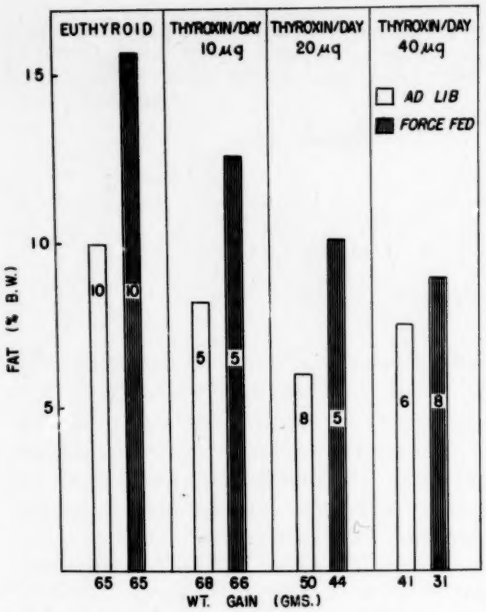

FIG. 2. Effect of exogenous thyroxin on body fats of normal rats. At the room temperature at which the animals were housed (25°C.), the endogenous secretion of thyroxin was considered to be 2 to 3 μg. per day.[15]

These experiments with pair gaining and pair feeding led us to the conclusions that the meal eater was more economical in its use of calories than was its nibbling control and that the unexpended calories were deposited as fat. The diet was approximately twice as efficient when it was meal eaten in contrast to when it was nibbled,[5] using as a test of efficiency of the diet, the calories retained in the body compared to the calories consumed.

In addition to our observations, van Putten and co-workers have reported the results of their studies which are consistent with our interpretation of the effect of the rate of food ingestion on body metabolism.[13] They noted that when rats with the hypothalamic-hyperphagic syndrome were pair fed against control animals, the hyperphagic ones ate their daily allotment of food in a short period of time but the control rats required twenty-four hours to eat their diet. The hyperphagic rats contained double the amount of carcass fat but less carcass protein than did the control animals. Restricting the time of food intake of both groups to an hour in the morning and to an hour in the afternoon, resulted in an equalization of the time of food consumption and almost complete equalization of the body composition of both groups. Feldman et al.[14] found that male chickens, made aphagic with diencephalic lesions and therefore requiring tube feeding, developed grossly increased quantities of subcutaneous fat.

Faced with what appeared to be a decrease in the expenditure of energy accompanying forced-feeding, it became necessary for us to evaluate a possible causative role of the thyroid gland in our findings. The resultant studies of rats revealed that meal eating was associated with decreased uptakes of I^{131} by the thyroid and that the lessened activity of the thyroid seemed to be secondary to a decreased rate of formation and/or release of the thyroid stimulating hormone.[7] The decrease in thyroid activity could not be attributed to handling, composition of the diet, presence or absence of the adrenals, body activity or to dilatation of the stomach.[7,15] Kalant and co-workers[16] not only confirmed the fact that the force fed animal exhibits a decreased ability to accumulate I^{131} in the thyroid but they also demonstrated that the release of I^{131} (and presumably thyroid hormone or hormones) from the thyroid proceeds at a decreased rate in animals fed in this way.

At this stage in our studies, we were confronted with two possibilities regarding the effect of meal eating on metabolic processes in the body. On the one hand, the data on thyroid activity suggested that the hypothyroidism associated with forced-feeding contributed to the increased retention of fat in the force-fed animal. However, our results did not disprove the hypothesis that the decreased thyroid activity might be secondary to metabolic adjustments resulting from the rapid ingress of food and, therefore, not involved in a cause and effect relationship to the increased body lipid. On the other hand, it was also clear to us that meal eating, with its peak loads of calories, might change the enzymatic machinery of the body to alternate rate-limiting pathways with subsequent changes in over-all body metabolism. To evaluate both

possibilities, the following were performed.

(1) Role of the thyroid in altered body composition. If the hypothyroidism in the force-fed rat was the cause of the increased body lipid, giving an excess of thyroxin to nibbling and force-fed rats pair fed against each other should have resulted in an equalization of body fat content provided that the dose of hormone was great enough to inhibit the release of endogeneous TSH and thyroxin. Accordingly, body lipids were determined in rats given 10, 20 or 40 μg. of thyroxin daily under the conditions of the two feeding regimens. Force feeding was still associated with increased body lipid, although at higher levels of hormone administration, the levels of body fat tended to approach each other (Fig. 2). The possibility that the endogenously secreted or the exogenously injected thyroxin was lost in the feces and therefore not available for its physiologic function[17] was negated by experiments with I^{131}-labeled thyroxin. The labeled thyroxin was injected into both the nibbling and force-fed rats and fecal and urinary excretions of I^{131} were determined. Regardless of the manner of feeding, the urinary and fecal losses of I^{131} were similar (Fig. 3).

(2) Role of the rate of ingestion of the diet on enzymatic activities. To test the possibility that meal eating may have resulted in changes in traffic over specific enzymatic pathways, when multiple alternate pathways were available, the activity of the hexosemonophosphate oxidative shunt was evaluated under the conditions of the two different methods of food intake. In addition, the measurement of shunt activity would provide information regarding the increased lipogenesis seen in the force-fed rat. Langdon has demonstrated that the shunt generates TPNH,[18] which is necessary in the present scheme of lipogenesis, for the conversion of crotonyl coenzyme A to butyryl coenzyme A, a rate-limiting reaction in fat formation. Furthermore, under some circumstances, there appears to be some correlation between shunt activity and lipogenesis. Using both the measurement of glucose-6-phosphate (+6-phosphogluconate) dehydrogenase activity in the super-

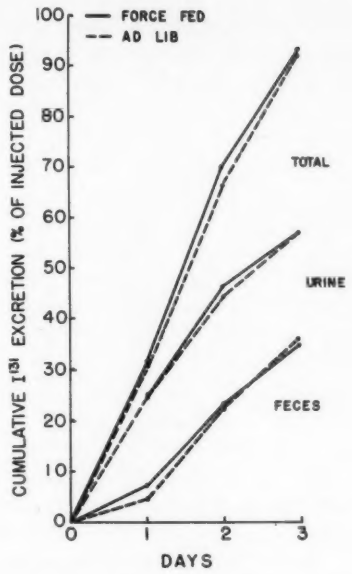

FIG. 3. Excretion of I^{131} in the urine and feces of nibbling and force-fed rats which had been injected with I^{131}-labeled thyroxin on day 0.[15]

natants of homogenates of liver and epididymal fat pads and the oxidation of C-6 and C-1 C^{14}-labeled glucose by liver slices *in vitro*, evidence was found that shunt activity was increased in the tissues of the force-fed animal.[6] The results are consistent with the conclusion that the rate of ingestion of food plays a role in regulating intracellular enzymatic activity with respect to both fat and carbohydrate metabolism.

The effect of the manner of eating on carbohydrate, fat and protein metabolism may be direct or mediated through endocrine influences. In support of this latter possibility, is evidence that meal eating alters endocrine gland activity *per se*. Thus, in addition to the aforementioned experiments on thyroid function and TSH formation and/or release thereof, we have observed a decreased rate of urinary 17-ketosteroid excretion in the force-fed rat, as compared to the ones eating *ad libitum*. These latter results could be attributed to decreased secretion by the pituitary of ACTH.

The rate of ingestion of food seems to play a part in the development of potential diabetes. Houssay has reported his findings on

rats with 95 per cent of their pancreas removed and given one or three meals a day.[19] The animals given one meal per day became diabetic before those which received the same amount of food in three feedings. Furthermore, a greater percentage of the rats given one meal per day developed the diabetic syndrome.[20] Additional data on meal eating and the enhancement of potential diabetes in rats is available from the laboratory of Engle.[21] The rat, which is usually resistant to the diabetogenic activity of the growth hormone, developed temporary diabetes when tube fed and injected with this hormone. One may speculate that the diabetes seen in the meal eating rat resulted from a greater hyperglycemia after meals, leading to exhaustion of the pancreatic islet cells or to interference in the formation of insulin as a result of meal eating. As it was previously pointed out, meat eating appears to inhibit protein synthesis in general; it is quite possible that protein hormone formation in particular also may be affected.

The animal kingdom may be divided into two different types with regard to eating habits—the meal eaters and the nibblers. In general, the meal eaters tend to be carnivors and the nibblers the herbivors or omnivors. The eating habits may be evolutionary vestiges and may be based on the availability of food. The laboratory rat is normally a nibbler and lends itself admirably to studies designed to explore the effects of the rate of ingestion of food on intermediary and whole body metabolism. When it is forced to eat meals, changes in the rate of absorption of the calories for the day appear to produce differences in the traffic over enzymatic pathways.

Instead of the slow semicontinuous ingression of foodstuffs to which the rat seems to be adapted under the usual laboratory conditions, it is flooded with and forced to dispose of calories on several "widely" separated occasions. The spaced loads of food may well alter the rate-limiting enzymatic steps. Therefore, it is not at all unexpected that the different eating habit, because of the known complexity of intermediary metabolic processes, may result in changes in over-all body metabolism

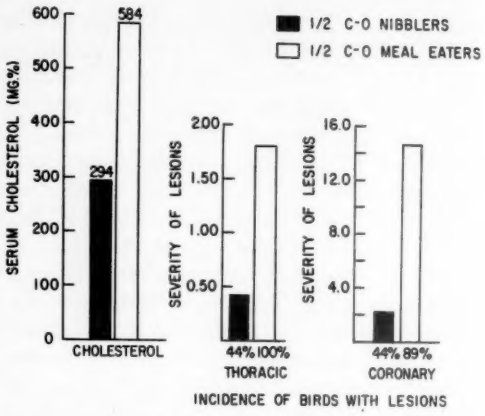

FIG. 4. Effect of meal eating or nibbling a 0.5 per cent cholesterol, 5 per cent fat, 20 per cent protein diet for five weeks on aortic and coronary artery atherogenesis in the chicken. Blood for determinations of cholesterol levels were drawn eighteen hours after the meal eaters had last had food while the nibblers had eaten all night.[24]

secondary to changes in specific reactions. Therefore, it might be anticipated that the different eating habits (meal eating versus nibbling) would be associated with changes in fat, carbohydrate and protein metabolism, in view of the known enzymatic interrelationships between the various food-stuffs. It should be pointed out that the differences in the economy of ingested calories between the two different types of eating appear to provide a means for the evaluation of thermodynamic principles as applied to open biological systems. The evidence indicating that it may be incorrect to apply classical thermodynamics, assumed to exist in closed systems, to biological systems was summarized recently.[22]

Assuming that the rate of ingestion of the diet plays a role in the regulation of body metabolism, might eating habits have an effect on the production and regression of metabolic diseases? The possibility that eating patterns may contribute to or aggravate a number of diseases characterized by abnormalities in fat, protein and carbohydrate metabolism was considered.

Studies were accordingly designed to explore the influence of the manner of eating on experimental atherosclerosis.[23,24] In the first

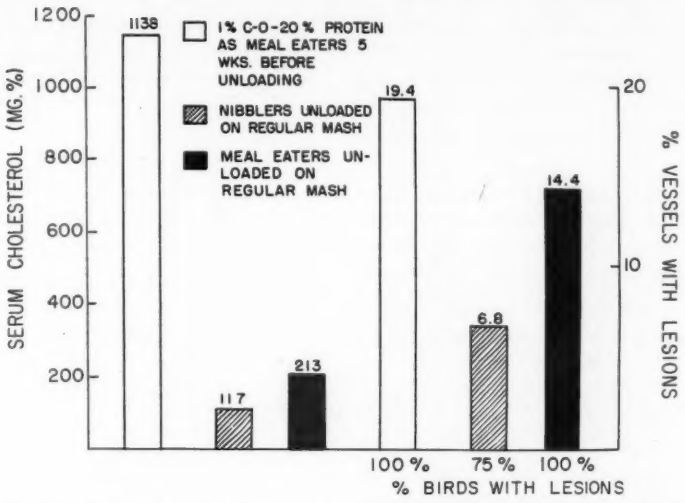

FIG. 5. Regression of coronary artery atherosclerotic lesions and serum cholesterol levels in chickens with established lesions. During the regression phase, the birds either nibbled or meal ate a cholesterol-free diet for two weeks.[24]

series of experiments, the effect of meal eating versus nibbling on the production of atherosclerotic lesions was investigated. A diet low in cholesterol was offered to chickens which had free access to food twenty-four hours per day (nibblers) and to those which received food for only an hour in the morning and an hour in the afternoon (meal eaters). Five weeks after the animals were placed on the diet and on their particular feeding regimen, they were sacrificed and their serums were analyzed for the concentration of lipids. The aortas and the coronary arteries also were examined in order to determine the number of and the severity of atherosclerotic lesions. It should be noted that during the feeding period, the meal eaters ate about one-third less of the diet and were, therefore, subjected to the atherogenicity of one-third less cholesterol. Furthermore, on the morning of sacrifice, eighteen hours after the meal eaters had last had food, in contrast to the nibblers which had eaten all night, the animals were bled. The data in Figure 4 indicate that meal eating was associated with seven times the incidence of coronary artery disease and with double the level of serum cholesterol.

In another type of experiment, the influence of eating habits on the regression of established atherosclerosis was studied. Chickens were trained to meal eat a moderately high cholesterol diet for five weeks. At that time, one-third of the birds (controls) were killed in order that examinations could be made of their coronary arteries for atherosclerotic lesions and of their serums for lipid concentrations. The surviving chickens were offered a cholesterol-free diet for an additional two weeks, under the conditions that one-half of the birds continued to eat the food as meals and the other half were allowed to revert back to the nibbling state and consume the diet in this way. At the conclusion of this two-week feeding regimen, the chickens were sacrificed and studied employing the same methods used with the control animals. Even though both groups of chickens had consumed a cholesterol-free diet for two weeks, the data in Figure 5 reveal that meal eating, when compared to nibbling, was associated with some degree of maintenance of the elevated serum cholesterol and was accompanied by a diminished ability to clear the coronary arteries of the atherosclerotic lesions.

The results of both of the studies of induction and regression indicate that the rate of inges-

tion of the diet does play a role in experimental atherosclerosis. Confirmation of these findings may be found in the report of Cox and co-workers;[25] we interpret their data to indicate that meal eating enhances atherogenesis in the monkey.

At this time, it appears of utmost importance to ascertain how man reacts to various eating habits in health and disease. At birth, man may be regarded as a nibbler, but a number of factors including convenience, habits and working conditions soon change him into a meal eater. Once the meal eating pattern is established, it persists because of custom. In epidemiologic studies of metabolic diseases, attention may be profitably directed toward eating habits. Although there have been numerous world-wide surveys to determine the relationship of atherosclerosis to dietary constituents, eating habits have been heretofore neglected.

A number of questions concerning the therapy of human disease are raised by the experimental results summarized. Might not diabetics be more easily controlled if they consumed their daily allotment of food in frequent small feedings? Is meal eating one of the connecting links in the relationship of diabetes to obesity and atherosclerosis? Might not patients with chronic hepatitis and those with chronic renal disease benefit from nibbling? Such a regimen might eliminate the problem of disposing of overwhelming loads of foodstuffs by the enzymatic systems of diseased organs which are probably already overloaded with substrates. Therapy of acute hepatitis, for instance, includes urging patients to eat candy or high carbohydrate foods constantly; might not the beneficial effects of this practice be attributable to nibbling, rather than to the high carbohydrate intake? In view of the diminished ability to store protein in the meal eating animal, might not diseases characterized by protein deficiencies be treated more efficiently if frequent small meals were prescribed? Might not both protein and vitamin requirements be less, with respect to amounts required for "balance," under the conditions of nibbling?

Thus it may be seen that man's reaction to meal eating and nibbling require clarification. The situation may well be summarized by the paraphrase, "To nibble or not to nibble, that *is* the question."

SUMMARY

Animals induced to meal eat (consume full spaced meals) differ from those allowed to nibble (eat frequent small feedings) with respect to over-all body metabolism. Meal eating, when compared to nibbling, is associated with the following: (1) increased body fat, (2) decreased body protein, (3) changed tissue enzymatic activities, (4) altered thyroid activity, (5) an increased incidence of diabetes mellitus in partially depancreatized rats and (6) an enhanced development of and an inhibition in the regression of experimental atherosclerotic lesions. These results are interpreted to be the result of a role the rate of ingestion of the diet plays in the regulation of intermediary metabolism. It is believed that the rate of influx of calories alters traffic over specific enzymatic pathways, when multiple pathways are available, hence it affects the metabolism of fat, carbohydrate and protein.

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DISCUSSION

DR. DONALD S. FARNER (*Pullman, Washington*): I have become more and more impressed in recent years with diurnal physiologic cycles. I am wondering, with respect to your experimental data, what would happen if rats were forced to become daytime nibblers or if, perhaps, they were force-fed at night. In other words, whether there is something in the physiologic cycle that gears the animal to receive food at a particular time. This is built-in genetically; now his food is becoming available at a different time.

What happens over a long duration? Do these force-fed rats continue to maintain the same difference in weight over the nibblers? In other words, do they become accustomed to force feeding and tend to slide back to similarity with the nibblers?

DR. COHN: With respect to the first question, we have debated this one ourselves, and I feel that perhaps sometime during the coming year, we should do the experiment of force feeding the rats at night. This has troubled me.*

Also as part of the first question, I discussed this with Dr. Fleming, who told me that when one attempts to adapt a rat to eating his own food for two hours a day it is much easier if this is done at night rather than in the daytime.

I have never been able to get our so-called trained rats, trained to eat for two hours a day, an hour in the morning and an hour in the afternoon, to gain weight at the same rate as do these fed *ad libitum*. Maybe others have been successful. If not, perhaps Dr. Fleming's suggestion of feeding at night might overcome this.

With respect to the second question, the longest that we have fed with this in mind has been about eight weeks. They do maintain the fat differential, but the increment in fat becomes less with time.

DR. A. QUERIDO (*Holland*): I was pleased with the presentation of Dr. Cohn's paper, for several reasons: first of all, this experiment of van Putten came from our group of investigator's, and we have been very worried since we performed it because nobody had ever confirmed it. This was a very difficult experiment, so we did not believe that we should repeat it ourselves. Now I have the satisfaction that the experiment was a valid one and has now found confirmation in another device and experiments. I think that, furthermore, this has increased its validity because of your finding of these differences in shunt.

The question I am putting to you is what it means, because we have had great difficulty in visualizing, actually, where it came from.

There are, of course, two possible explanations. The first is that the two groups of animals have a great

* Since the Conference, we have force-fed our rats at night and found results similar to those obtained with force feeding animals in the daytime; namely, the force-fed rats have more body fat than the ones eating *ad libitum*.

difference in total energy expenditure and, therefore, this difference in fat content is manifest. The other possibility, however, is that there is a greater efficiency of handling calories in an animal that is force-fed versus an animal that is space-fed.

We have been trying to make various schemes whereby we have tried to figure out how much of an energy package, in the form of ATP or anything else, could be produced if you had a different scheme. Of course, we did not know at that time the shunt. Assuming that the total expenditures are identical in the two groups, the making of fat must have cost a great deal of energy and, the fat is being disposed of, probably, at a time when the animal does not eat to give the fuel package. This would mean that there is a terrific difference in the efficiency of release of energy from the fat stores in comparison to release of energy from the food that was available during the meals.

If I have expressed myself clearly on this problem, I would like very much to hear whether you have thought about it, whether you have tried to analyze these pathways in such a way that have an acceptable explanation for this difference—assuming, of course, that the total expenditures are identical.

DR. COHN: We attempted to perform one experiment which gave us the opposite result of what we had planned. Dr. Ingle told me why. We put rats in restraining cages in an effort to control expenditure of energy, and then fed them *ad libitum* or force fed them. I think this was the best exercise experiment we have ever done, since, as Dr. Ingle has pointed out, unless one slowly adapts the animals to the cages, they really struggle. They gained weight only at the rate of about 50 per cent of what we usually anticipate. As I say, the energy loss in these animals from their exercise must have been tremendous.

Under these conditions, the force-fed animals still had double the amount of body fat of those fed *ad libitum*.

I agree with you that somewhere in the body economy the handling of the ingested calories is influenced by the rate at which the calories become available to the animal from the gastrointestinal tract.

DR. WILLIAM PARSON (*Charlottesville, Virginia*): Dr. Cohn, I would be interested to know how soon you could show changes when you compared your force-fed rats with the rats fed *ad libitum*.

Stimulated by one of your earlier reports and by some of the studies of the Teppermans, Dr. Holifield, in our laboratory, has studied the rate of synthesis of fat of the epididymal pad, using C^{14}-acetate, following the training of rats to eat in two-hour periods daily. Despite the fact that our animals did not maintain weight, we could show a stepwise increase in the rate of synthesis of fat, reaching a peak in five days and then maintaining a plateau at that point. Associated with this increased synthesis of fat was an increase of the monophosphate shunt, using the technics that you have described.

DR. COHN: You have done an experiment which we are planning to do.

DR. PARSON: We have not reported it as yet.

DR. JOHN R. BROBECK (*Philadelphia, Pennsylvania*): I wonder whether you would consider not using the terms "nibbler and meal eater," considering the fact that rats normally do eat meals, but they eat more meals at shorter intervals than is desirable for purposes of this experiment. What you have done, and what Dr. Tepperman did, has been to oblige these rats to eat meals at the intervals that human beings do, rather than at the intervals rats prefer.

I do not want to detract in any way from these experiments, but it might be worth considering the use of different terms.

DR. COHN: The terminology that we are employing was occasioned by the fact that in contrast to human beings, rats do have more frequent but smaller meals. This was the only reason for a recourse to nibbling.

DR. BROBECK: I have another reason for mentioning this. This has often confused studies on dogs. In some of the experiments reported by Dr. Grossman in which food intake was measured in dogs fed once a day, I believe he not only had animals that were utilizing their food abnormally, as you would suggest, but the regulation was changed by the way in which they were fed. I think if one would study regulation in dogs fed twenty-four hours—as you call them, nibblers—rather than in dogs fed just one meal, one might get different results in the timing of some of these responses.

Some Hormonal Influences on Fat Mobilization from Adipose Tissue

FRANK L. ENGEL, M.D.* AND J. EARLE WHITE, JR., M.D.†

THE IDENTIFICATION of albumin-bound free fatty acids‡ as a major transport form of lipid has introduced a new parameter into the study and understanding of lipid mobilization.[1,2] It now seems to be well established that adipose tissue is the major if not the only source of these free fatty acids which, in turn, are available for utilization by other tissues including those of the skeletal and cardiac muscles and the liver.

These observations have made possible the development of several new approaches to the elucidation of the roles of the nervous and endocrine systems in lipid mobilization. Earlier approaches depended largely on the measurement of changes in lipid content of appropriate adipose tissues under different experimental conditions and on the production of fatty liver.[3,4] The latter has been interpreted as reflecting lipid mobilization from peripheral depots, but this assumption has not always been justified.

The influence of various nutritional and hormonal factors on levels of plasma free fatty acids in man and animals was scrutinized in several laboratories, notably those of Dole and of Gordon and Frederickson, once the analytic technics were established and the source and metabolism of these fatty acids were elucidated. Certain inferences could be made concerning the roles of nutritional factors and hormones in fatty acid mobilization, by combining measurements of plasma fatty acid concentration with those of arterio-venous differences and with the estimation of the specific activity of constantly infused C^{14}-palmitate in plasma. In a general way, it was concluded that factors which lead directly or indirectly to increased carbohydrate utilization inhibit the net release of fatty acid from tissue stores, while conversely, conditions associated with impaired glucose utilization accelerate the release of fatty acid.[1,2]

The first direct estimates of lipid mobilization *in vitro* were made independently by Gordon and Cherkes[5] and White and Engel.[6,7] Both groups estimated the release from adipose tissue of free fatty acids and their binding to albumin in an appropriate *in vitro* incubating medium. The former group demonstrated that adipose tissue removed from fasted rats released more free fatty acids than did that from fed animals, and that glucose and insulin in the medium inhibited fatty acid release while epinephrine stimulated it. The latter, using only adipose tissue (incubated in rat plasma) from fasted rats, reported that epinephrine, norepinephrine and corticotropin had potent lipolytic activity, while growth hormone and thyrotropin (TSH) had modest activity. Free fatty acids accumulated in the tissue, before they appeared in the medium, in response to these hormones. It was concluded that the

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‡ These long-chain fatty acids are also referred to as non-esterified fatty acids (NEFA) and unesterified fatty acids (UFA).

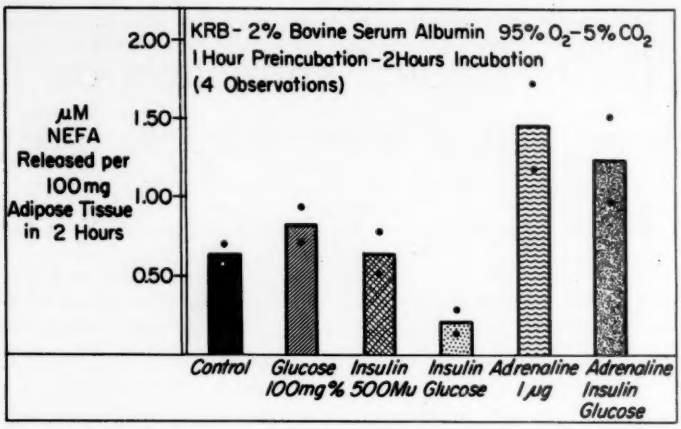

FIG. 1. Influence of insulin and epinephrine on fatty acid release by adipose tissue *in vitro*. In all figures, KRB = Krebs-Ringer bicarbonate medium; Mu = milliunits.

hormones influenced the hydrolysis of triglycerides fairly directly and perhaps activated lipase in the adipose tissue. Since these initial reports were made, the basic findings therein have been confirmed and considerably extended in many different laboratories. In this report we have reviewed the present status of the problem of hormonal control of lipid mobilization from adipose tissue with particular reference to the correlation of the results found *in vitro* and *in vivo*.

EPINEPHRINE, NOREPINEPHRINE AND THE SYMPATHETIC NERVOUS SYSTEM

Studies carried out long before the current enthusiastic interest in a possible neurohumoral control of the metabolism of adipose tissue demonstrated that denervation of adipose tissue compromised its ability to mobilize its lipid in response to fasting and other stimuli.[3,4,8,9] Although no specific inferences can be drawn from these studies as to whether or not adipose tissue innervation is autonomic, this conclusion is suggested by the recent studies which demonstrated that epinephrine and norepinephrine, when infused intravenously, cause an increase in the levels of plasma fatty acid and also lead to lipolysis and a release of free fatty acid from adipose tissue incubated *in vitro*.[1,2,6,10-12] The infusion of norepinephrine leads to a more sustained increase in plasma fatty acids in man than does epinephrine.[10] In the latter case, it is probable that hyperglycemia resulting from the infusion of epinephrine eventually stimulates a compensatory secretion of insulin. Insulin effectively inhibits fatty acid discharge. Goldfien and Havel found that hexamethonium administered to dogs lowers the rises in plasma fatty acids induced by epinephrine, norepinephrine and fasting.[10] Bogdonoff reported that, in man, Arfonad® (trimethaphan camphorsulfonate) inhibits the rise of free fatty acid which occurred in response to psychologic stress and infusion of norepinephrine.[12-13b] In the rat, Goodman and Knobil failed to demonstrate any effects of the administration of ergotamine, Dibenzyline® and hexamethonium on the levels of plasma fatty acid during fasting.[14] However, Wertheimer et al. reported that pretreatment of rats with Dibenzyline inhibits the subsequent release of fatty acids from incubated adipose tissue removed from fasting, diabetic, cold-exposed, endotoxin-treated and hyperthyroid rats.[15] Schotz and Page claim that Regitine®, at a concentration of 10^{-3}M, completely blocks the release of fatty acid from adipose tissue incubated with 10^{-1}M epinephrine or norepinephrine.[11]

The results of these studies lend strong support to the concept that the release of fatty acids from adipose tissue is under tonic control

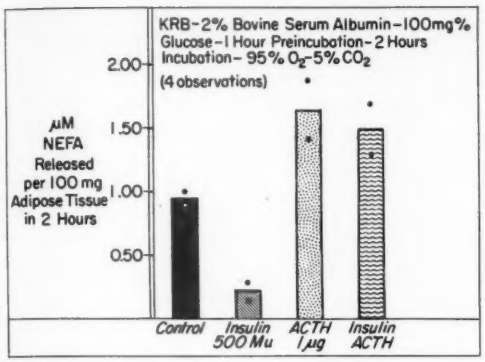

FIG. 2. Influence of insulin and ACTH on fatty acid release by adipose tissue *in vitro*.

of the nervous system, in all likelihood via the release of norepinephrine from the nerve endings. There is an urgent need for assays to be made of the content of norepinephrine in adipose tissue. Examination of Sidman and Fawcett's microphotographs of nerve fibers in adipose tissue certainly demonstrates a richness of distribution which may be surprising to those who had not previously given much consideration to innervation in adipose tissue.[16]

The possible mechanisms by which epinephrine and the other hormones induce fatty acid release from adipose tissues will be discussed later. Wadstrom showed that the concentration of lower glycerides increases in the adipose tissue of rabbits treated with epinephrine[17]; we found that extractable free fatty acids increase to a measurable level within incubated adipose tissue before they increase in the medium[7]; Cahill,[18,19] Lynn[20] and their associates demonstrated the liberation of glycerol from hormone-stimulated adipose tissue *in vivo* and *in vitro;* all of these studies point to hydrolysis of triglycerides as a key reaction, but do not distinguish between primary and secondary control of this step.

INSULIN

The administration of either insulin or glucose depresses free fatty acid levels and decreases fatty acid arterio-venous differences in the plasma of normal man and animals. Glucose is ineffectual in those with diabetes unless insulin is administered simultaneously.[2] Bierman et al.[21] have shown in dogs that during a constant infusion of C^{14}-palmitate, specific activity of plasma fatty acids rises as the concentration of unlabeled fatty acids falls in response to insulin. They therefore concluded that insulin inhibits net fatty acid release from adipose tissue, which accounts for the increase in the specific activity of constantly infused palmitic acid in plasma. During fasting, plasma free fatty acid levels in diabetic patients rise much more rapidly than they do in normal subjects. The same result follows discontinuance of therapy with insulin. The ensuing rise in levels of free fatty acids precedes ketonemia.[22]

Wertheimer et al.[15] reported that adipose tissue from diabetic rats releases more fatty acids into the incubating medium than does such tissue from normal animals. Adipose tissues from normal fed rats contribute less fatty acids to the incubating medium than do tissues from fasted animals.[5] Addition of glucose to the incubation medium reduces fatty acid release and accumulation in the tissue.[23] Insulin accentuates the reponse to glucose but it has no measurable influence in the absence of glucose in the medium (Fig. 1). These findings suggest that the action of insulin on fatty acid balance of adipose tissue is secondary to its influence on uptake and utilization of carbohydrate from the medium. The data of Cahill[18] and of Lynn et al.[20] from Duke University support this conclusion.

Insulin increases the utilization of glucose via all available pathways, including formation of triglyceride fatty acid, with the net balance favoring retention of lipid. Although only small amounts of labeled palmitate infused into dogs and rats could be accounted for as triglyceride fat,[1] Cahill,[18] Lynn[20] and Raben and Hollenberg[24] found that glucose and insulin promote the uptake and presumed incorporation of C^{14}-fatty acids into triglycerides of adipose tissue incubated *in vitro*. In our experience the fatty acid mobilizing effects of epinephrine and ACTH have generally outbalanced the opposite influences of insulin and glucose on the fatty acid accumulation in and release from adipose tissue of fasted rats (Figs. 1 and 2). Quantitatively, the effects of insulin and

glucose on fatty acid release are small compared to those of epinephrine and ACTH, suggesting that the former are acting on one or a number of interconnected reactions which are indirectly related to the lipolytic reaction itself, whereas, ACTH and epinephrine influence triglyceride hydrolysis more directly.

ANTERIOR PITUITARY GLAND

This gland has been implicated in lipid mobilization and metabolism for a long time, and these actions have generally been attributed to growth hormone.[25] However, other anterior pituitary hormones (corticotropin and thyrotropin), even in the absence of their target glands, also have effects on lipid metabolism comparable to those of growth hormone.[26] With ACTH the evidence suggests that these extra-adrenal activities are due to corticotropin itself and not to a contaminant, but firm proof for this conclusion is still lacking. With TSH, on the other hand, Steelman has evidence that the lipid mobilizing (adipokinetic) activity of TSH may be dissociated from its thyroid-stimulating activity.[27] There are claims, which need to be substantiated, that the pituitary gland secretes a factor separate and distinct from the known hormones, which influences lipid metabolism.[28] The significance of the apparent common metabolic activities of different pituitary hormones is discussed elsewhere.[26]

Levels of plasma free fatty acid increase during fasting to approximately the same degree in hypophysectomized monkeys as in normal monkeys,[29] even though adipose tissue removed from hypophysectomized rats releases fatty acids more slowly into the incubating medium than does tissue from normal rats.[30] However, the normal plasma fatty acid response to fasting of the hypophysectomized animal may be more apparent than real. In view of the greater tendency toward hypoglycemia during fasting after hypophysectomy, the metabolic stimulus to lipid mobilization should be greater than normal at any given point along the time scale of fasting. Amatruda and Engel report that hypophysectomized rats exhibit significantly greater ketonemia during fasting than do normal rats, but are correspondingly more hypoglycemic. When a comparable degree of hypoglycemia is induced in fasting rats by treatment with phloridzin, greater ketosis than is found in the hypophysectomized animals results, indicating that in the absence of the hypophysis there is in fact an impaired ability to develop ketosis.[31] Presumably, pituitary hormones are not essential for fatty acid mobilization and ketosis even though they may be required for the optimal response. Administration of minute doses of simian and human growth hormone leads to impressive and prolonged rises in the levels of plasma fatty acid in hypophysectomized monkeys and man. Administration of larger doses is required in intact animals, but the diabetic dog also responds to small doses.[29,32–35]

Although the administration of growth hormone also increases plasma fatty acids in the rat,[36] this response is not as predictable and reproducible as it is reported to be in other species. We have treated normal rats with large doses of growth hormone for as long as eight days without detecting any consistent changes in plasma fatty acids during the course of treatment. Pretreatment of hypophysectomized rats with growth hormone partially restores the ability of adipose tissue to release fatty acids *in vitro*.[30] Different batches of bovine, simian and human growth hormone, incubated in concentrations of from 10 to 500 μg./ml. *in vitro* with adipose tissue from normal rats, have exhibited definite lipolytic activity.[7,37] Growth hormone is not as inactive in this system, as has been implied in several recent publications,[29,35] although admittedly the amounts of hormone are much greater than those required to elevate levels of plasma fatty acid *in vivo*.

From these data one might question whether any hormone is required for the plasma fatty acid response to fasting in the hypophysectomized animal. Even epinephrine has been found to be surprisingly ineffectual when injected into hypophysectomized monkeys.[38] Treatment with the growth hormone, but not ACTH, cortisone or prolactin, restored the ability of the hypophysectomized animal to respond to epinephrine. However, it was

demonstrated that an amount of TSH, estimated to contaminate the growth hormone used, as well as triiodothyronine were as effective as growth hormone in restoring the response to epinephrine.[29] Presumably the TSH contamination was responsible for the observed effects of growth hormone. Subsequently, other investigators have reported adipose tissue of hypothyroid animals also to be unresponsive to epinephrine *in vitro*.[39] Restoration of the euthyroid state re-established the lipolytic response of the adipose tissue while hyperthyroidism accentuated it.[40]

At the present time, it may be concluded that while levels of plasma fatty acid may increase in response to fasting without intermediation of the hormones currently implicated in fatty acid release from adipose tissue, a role for these hormones physiologically is not thereby vitiated. Levels of plasma fatty acid and *in vitro* release of fatty acids represent a balance between a number of opposing metabolic processes, which may be differentially influenced by various hormones. The fasting hypophysectomized animal suffers from relative insulin deficiency and this defect by itself might contribute to a net accumulation of fatty acids even though there are other retarding influences, such as hypothyroidism. A similar explanation for the fasting during ketosis of hypophysectomized rats was suggested. This can be promptly abolished by the administration of a dose of insulin which is so minute that it does not lower further the already depressed levels of blood sugar.[31]

As noted already, other pituitary hormones besides growth hormone have been reported to influence adipose tissue metabolism and plasma fatty acid levels. The action of TSH in the hypophysectomized animal has just been described and is attributable to the stimulation of thyroid hormone secretion. TSH itself also acts on adipose tissue *in vitro*[7] but in view of the impurity of the available preparations of TSH, this action cannot yet be attributed specifically to TSH. The same considerations apply to our finding that a sample of follicle-stimulating hormone (FSH) had lipolytic activity *in vitro* at a concentration of 10 μg./ml.[37] Prolactin, α and β-melanocyte-stimulating hormone (MSH), and synthetic lysine vasopressin have been found inactive in the *in vitro* system in concentrations up to 100 μg./ml. in this laboratory.[7]

The most active pituitary peptide in inducing fatty acid release from adipose tissue *in vitro* is corticotropin.[7,18,20,41,42] Curiously, however, this hormone has been impotent when assayed for its effects on the levels of plasma fatty acid *in vivo*,[33,37] even though the same hormone may induce ketosis and fatty liver in rats and mice[43,44] and accelerate the loss of lipid during fasting from the paired epididymal fat pads of mice.[45a] This discrepancy might yet be reconcilable when it is appreciated that the discordant measurements have been made on animals of different species. Ketosis, fatty liver and liquid loss from adipose tissue *in situ* have been observed in rats and mice. Most of the studies on plasma fatty acids have been made on dogs, monkeys and man. We have been unable to induce ketosis in dogs with the administration of ACTH.[37*]

As with epinephrine, the net effect of ACTH on fatty acid release from adipose tissue *in vitro* is reduced in the presence of glucose or glucose and insulin in the medium but never obliterated (Fig. 2). We have found that the effect of ACTH on lipolysis always outweighs the opposing influence of glucose and insulin in reducing net fatty acid release. Even when the adipose tissue is preincubated with insulin, washed and then transferred to new medium to which ACTH has been added, the effect of insulin on fatty acid accumulation is largely abolished (Fig. 3). Under the conditions of this experiment, ACTH must compete favorably with insulin for any presumed binding sites.

ACTH, like epinephrine, also has striking effects on the carbohydrate metabolism of adipose tissue *in vitro*.[18,20] Utilization of glucose is stimulated by ACTH in certain circumstances while in others uptake is inhibited (Fig. 3).[46] The significance of these findings is discussed later as well as reviewed in other reports.[15,18]

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* Hollenberg et al. recently reported an *in vivo* effect of ACTH on plasma fatty acids in the rat.[45b]

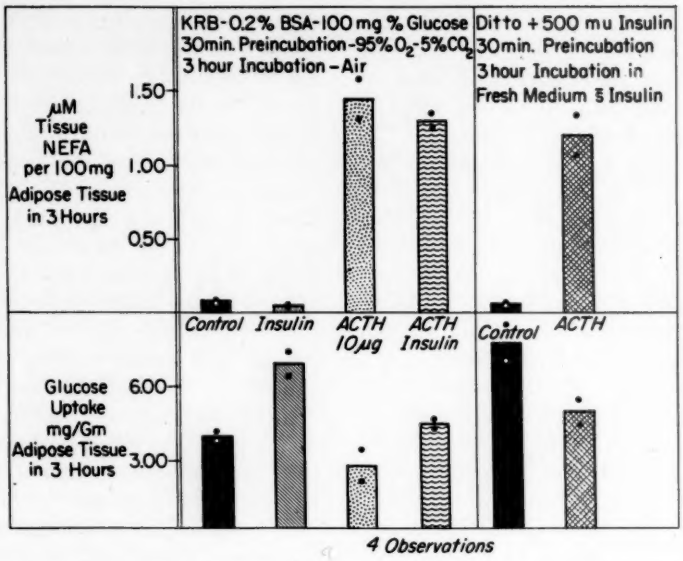

FIG. 3. Interaction of ACTH and insulin on adipose tissue fatty acid content and glucose uptake. In the panel at the left, tissues were preincubated for thirty minutes in medium without insulin and then transferred to fresh media containing no hormones (the control medium), 500 mU. insulin, 10 μg. ACTH and ACTH and insulin. Note the stimulation of glucose uptake and the inhibition of fatty acid release by insulin and the reversal of these effects with ACTH. In the right panel the tissues were preincubated in medium containing 500 mU. of insulin for thirty minutes and then transferred to new medium containing either no hormone or 10 μg. ACTH. Note the persistence of insulin effect when tissue was transferred to fresh medium without insulin and the blocking of this effect by ACTH. BSA = Bovine serum albumin.

TABLE I

Recorded Extra-Adrenal Actions of ACTH*

1. Increase in liver lipid (adipokinetic).[43,47]
2. Mobilization of lipid from adipose tissue *in vivo*.[45]
3. Lipolytic, adipose tissue *in vitro*.[18,20,41,42]
4. Ketosis.[44,47]
5. Depression of respiratory quotient.[47]
6. Hypoglycemia in fasted intact adrenalectomized animals.[47,48]
7. Insulinotropic.[49]
8. Glucose uptake by adipose tissue *in vitro*.[18,20,46]
9. Activation of phosphorylase in adipose tissue *in vitro*.[20,41]
10. Increase in cardiac glycogen.[47]
11. Potentiation of steroid diabetes in force-fed, adrenalectomized rat.[37]
12. Decrease in urea formation from infused amino acids in adrenalectomized-nephrectomized rats.[50]
13. Melanophore stimulation.[47]
14. Inhibition of steroid glucuronide formation by liver *in vivo* and *in vitro*.[51,52]
15. Influence on renal excretion of corticosteroids.[15]

* This table includes all recorded studies as of 1959.

These effects of ACTH on adipose tissue *in vitro* are the most striking examples of extra-adrenal actions of this hormone so far described. In a previous report, we have described other extra-adrenal actions of ACTH and considered some of their implications.[26] Table I summarizes those which now seem reasonably well documented. From the results obtained to date (both in this laboratory and in others), the evidence that these various effects are due to ACTH, and not to a contaminating hormone or hormones or to some artifact, is strong, but not incontrovertible considering the uncertainties about the absolute purity of available samples of ACTH.

The evidence for the action of ACTH on adipose tissue is summarized herein, remembering that a number of other pituitary hormones and catechol amines have also been effective in this system.

(1) The activity of ACTH has always been considerably greater than that of any other pituitary hormone tested. Thus, most preparations of ACTH have been active at a concentration of 0.1 μg./ml., but some have been more potent. The smallest active dose of growth hormone and TSH has been 10 μg./ml. Vasopressin and α and β-MSH have been inactive in concentrations up to 100 μg./ml. These compare with epinephrine and norepinephrine which are consistently effective at 0.1 μg./ml.

(2) Several samples of ACTH of the highest degree of purity (i.e. corticotropin A from the Armour Company and A_1 from Dr. H. B. F. Dixon of Cambridge University) have been potent. One of the latter, assaying 70 USP units/mg., induced lipolysis at a concentration of 1×10^{-4} μg./ml.

(3) Incubation of ACTH in 0.1N solution of NaOH at a temperature of 26°c. from eighteen to twenty-four hours does not destroy adrenal-stimulating or extra-adrenal actions of ACTH, including lipolysis; whereas in most cases tested, growth hormone activity is lost. More vigorous treatment with alkali (i.e. boiling from fifteen to twenty minutes) inactivates ACTH in all respects except for its MSH activity, which increases.

(4) The adrenal-stimulating and the lipolytic and other extra-adrenal actions of ACTH have been either significantly reduced or abolished by treatment with H_2O_2 whereas growth hormone is unaffected by this treatment. Reduction of the inactive ACTH with cysteine restores activity. Alpha and β-MSH are also reversibly inactivated by H_2O_2 and cysteine, but, as previously noted, these hormones are impotent in the adipose tissue system.

(5) Calcium ion has been found to be essential for optimal steroidogenic action of ACTH on adrenal cortex slices *in vitro*.[53a] A similar requirement for Ca^{++} has been found by Lopez et al. for the lipolytic effect of ACTH[23] and by Verner and Engel for the action of ACTH on glucose uptake by adipose tissue *in vitro*.[37] Epinephrine is active in these systems regardless of the Ca^{++} concentration of the medium, indicating a specific role for Ca^{++} with respect to ACTH.

Taken as a whole, it is difficult to conceive of one of the accepted pituitary hormones being a contaminant in all our samples of ACTH and responsible for the actions of ACTH on adipose and other extra-adrenal tissues. Conversely, the low lipolytic activity of at least one sample of growth hormone tested was probably not due to ACTH since it resisted H_2O_2 treatment. Furthermore, other investigators have found injected growth hormone but not ACTH to be highly active in raising the levels of plasma fatty acid. If the effect which is thought to be caused by ACTH is actually due to some other peptide, then the latter must be a compound with a structure similar to ACTH, sharing many of its chemical properties.

Recent studies of corticotropin treated with periodate and periodate-borohydride suggest the possibility of the existence of peptides which are closely related to ACTH and which act upon adipose and extra-adrenal tissues without major influence on the adrenal cortex. One such peptide has been prepared and kindly supplied to us by Dr. H. B. F. Dixon of the University of Cambridge; it differs from ACTH only by lacking the N-terminal serine. Preliminary studies with a sample of the "periodate ACTH" (which, unfortunately, was too small to permit accurate quantitative comparisons) showed that its potency fell from 100 units/mg. to 2 to 3 units/mg., when assayed by the adrenal ascorbic acid depletion and plasma corticosterone methods in hypophysectomized rats, and from 39 to 5.4 units/mg. in the Saffran-Schally steroidogenesis assay* *in vitro*. In contrast, extra-adrenal activities, including effects on adipose tissue, persisted to a significant degree, albeit reduced. Unfortunately, periodate ACTH is quite unstable in solution.

More recently a new sample of periodate-borohydride ACTH, assaying <0.1 U.S.P. unit/mg., has been tested and compared with the untreated corticotropin A_1 (70 U.S.P. units/mg.).[53b] To our astonishment, this periodate-borohydride ACTH had unequivocal lipolytic activity at a concentration of 1×10^{-3} μg./ml. and borderline activity at a lesser concentration.

* We are indebted to Dr. R. Guillemin for performing these assays.

One $\times 10^{-4}$ μg./ml. of the untreated corticotropin was effective. This periodate-borohydride ACTH was more stable than the former periodate ACTH. If these results are confirmed and extended to other metabolic parameters, they may represent the first example of structural and functional dissociation between adrenal and extra-adrenal activities of ACTH. They suggest that the N-terminal serine of ACTH might be required for the specificity of the hormone for the adrenal gland while another part of the molecule is responsible for initiating the metabolic response to the hormone. The latter presumably, then, is non-specific. It may be noted that Ca^{++} is required for the lipolytic effect of both ACTH and periodate-borohydride ACTH, eliminating an interaction between the Ca^{++} and N-terminal serine.[37] An alternative explanation of the results obtained with corticotropin A_1 and its periodate-borohydride treated derivative is suggested by the fact that while the several samples of corticotropin A_1 tested have had comparable adrenal-stimulating potencies (70 to 100 units/mg.) and have been correspondingly inactivated by periodate-borohydride, their minimal active concentrations to affect adipose tissue have varied between 0.1 and 0.0001 μg./ml. This raises the possibility of the hormones being contaminated by an exceedingly potent peptide, which is not destroyed by periodate, despite the evidence for the chemical purity of ACTH and the evidence previously marshaled against the contamination of ACTH. Studies concerning this possibility are in progress, but so far do not support this interpretation.

ADRENAL CORTEX

To date not much has been reported on the relation of the adrenal cortex to the levels of plasma fatty acids and to the release of these acids from adipose tissue *in vitro* although a considerable body of data is available concerning the mobilization of fat to the liver. Adrenalectomy markedly retards the adipokinetic response to such agents as ethionine, carbon tetrachloride or ethyl alcohol.[54–57] According to Goldstein, Wool and their collaborators, treatment with cortisone is not sufficient to restore this response to normal, as epinephrine is also needed.[54,55] Mallov has recently questioned whether or not the lesser accumulation of liver fat in the ethanol-treated adrenalectomized rat is due to decreased mobilization of lipid in the periphery of the liver. He presents evidence that there might be an increased rate of fat catabolism in the livers of adrenalectomized rats.[57] However, Masoro was unable to detect any difference in the production of $C^{14}O_2$ from infused C^{14} palmitate-albumin in normal and adrenalectomized rats.[58] Two recent studies made on rats and mice, respectively, have demonstrated that lipid is lost during fasting more rapidly from the paired epididymal fat pads in adrenalectomized animals than from those in normal animals.[45,59] This difference is abolished by treatment with cortisone.

During fasting, levels of plasma fatty acid rise in animals upon which adrenalectomies have been performed, but not as rapidly as in normal animals.[14] However, administration of epinephrine does not increase the levels of plasma fatty acid in adrenalectomized rats, in fact, it may even lower them. Furthermore, adipose tissue from adrenalectomized rats does not respond readily to epinephrine[60] or to ACTH *in vitro*.[42] At this time it is not easy to reconcile these results with those of the studies on fatty liver and on lipid loss from adipose tissue *in vivo*. To date, data on the effects of overdosage of corticoid on lipid mobilization from adipose tissue have not been published.

COMMENTS

Studies on nutritional and hormonal factors influencing the levels of plasma fatty acid suggest a rather simple reciprocal relationship between glucose utilization and fatty acid release from adipose tissue.[1,2] However, certain observations of adipose tissue *in vitro* make it difficult to explain the hormonal stimulation of fatty acid release solely as secondary to changes in carbohydrate metabolism, even though these changes contribute to the net fatty acid balance. Our studies lead us to believe that ACTH and epinephrine must have primary actions on the hydrolysis

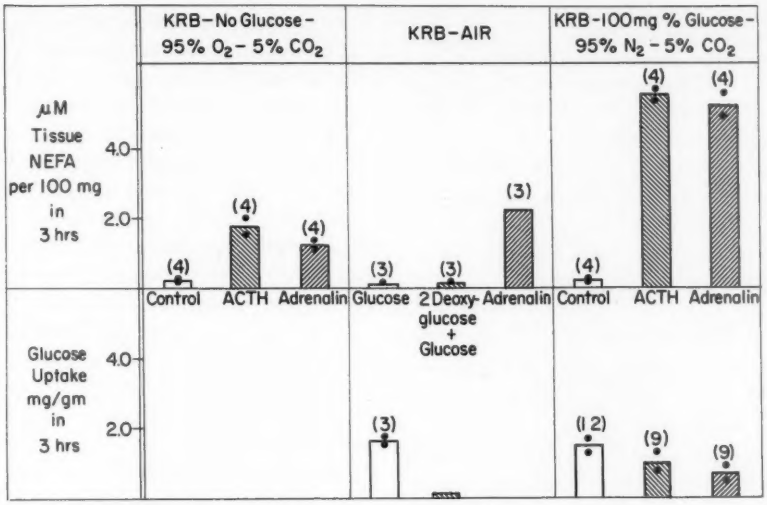

FIG. 4. Dissociation between lipolysis and glucose uptake in adipose tissue. Glucose and 2-deoxyglucose were added to a concentration of 100 mg. per cent and epinephrine and ACTH 10 μg./ml. KRB.

of triglycerides quite aside from any other influences on carbohydrate metabolism. Thus, if impaired glucose utilization were the main stimulus to fatty acid release, one would anticipate that adipose tissue from fasted animals would exhibit a vigorous release of fatty acid when incubated in a glucose free medium or when glucose uptake from the medium is blocked by such inhibitors of carbohydrate metabolism as 2-deoxyglucose. Figure 4 shows that this is not the case. Fatty acid release under these circumstances is modest, but it is greatly enhanced by the addition of epinephrine. It can be assumed that adipose tissue from the fasted rats has negligible glycogen stores. Wertheimer's finding that adipose tissue from diabetic rats pretreated with Dibenzyline® has only a modest rate of fatty acid release,[15] likewise implies that lipolysis, due to the diabetic defect, is not great but is magnified by the tonic effect of the catechol amine hormones. Figure 4 also shows that under anaerobic conditions, the release of fatty acid in response to ACTH and epinephrine, is increased out of proportion to the small decrease in glucose uptake. (The previous report that anaerobiosis prevented the lipolytic effect of ACTH[7] was in error, possibly because the pH of the plasma medium changed during incubation.) Lynn has also shown that iodoacetate and other poisons affecting carbohydrate metabolism do not enhance lipolysis.[20]

In a preliminary publication we reported that ACTH and epinephrine inhibited the uptake of glucose by adipose tissue *in vitro* and the action of insulin thereon, while at the same time they promoted fatty acid release (Fig. 3).[46] These results are at variance with those of Cahill,[18] Lynn[20] and their associates, who regularly have found that these hormones promote the uptake of glucose by adipose tissue. However, on comparing the experimental conditions under which the incubations were carried out in the several laboratories, a number of differences became apparent which presumably account for the discrepancies. Cahill et al. incubated adipose tissue in a Krebs-Ringer bicarbonate medium, containing glucose and 3.5 per cent bovine albumin, gassed with 95 per cent O_2 and 5 per cent CO_2. Under these conditions we have also found accelerated glucose uptake by tissues stimulated with ACTH and epinephrine. In our original experiments on glucose uptake, the Krebs-Ringer bicarbonate medium contained only 0.2 per cent albumin. Glucose uptake was

TABLE II

Relation of Fatty Acid Content of Adipose Tissue to Glucose Uptake During Stimulation by Epinephrine*

System		Epinephrine	Glucose Uptake (mg./gm. tissue)	Fatty Acid (μM/gm.)
Preincubation	Incubation			
O	O	None	1.06 ± 0.08	3.4 ± 0.15
A	O			
O	A	None	1.49 ± 0.10	1.6 ± 0.11
A	A			
O	O	10 μg.	0.69 ± 0.13	28.7 ± 0.80
A	O	10 μg.	1.87 ± 0.19	13.5 ± 2.40
O	A	10 μg.	2.02 ± 0.17	6.1 ± 1.10
A	A	10 μg.	3.24 ± 0.42	4.1 ± 1.20

NOTE: Fifty to 60 mg. fragments of adipose tissue were removed from twenty-four–hour fasted rats and placed in 1 ml. of Krebs-Ringer bicarbonate medium with 100 mg. per cent glucose without (O) and with 4 per cent bovine serum albumin (A). Ten μg. of 1-epinephrine were added to each flask except the control vessels. They were preincubated for thirty minutes at 37°c. in a Dubnoff incubator. At the end of preincubation the tissues were removed, transferred to new medium (with or without albumin) and incubated for an additional two and a half hours. Glucose utilization and tissue fatty acid concentration were estimated at the end of the incubation. There were eight observations for the experimental groups and four each for the control groups. The latter were pooled for the same "incubation" (AO, OO and OA, AA) since there were not significant differences between these. Results are expressed as mean ± standard error.

* Blackard, W. and Engel, F. L. Unpublished data.

usually depressed by the same hormones, whether the gas phase during incubation was air, 95 per cent O_2 and 5 per cent CO_2, 95 per cent air and 5 per cent CO_2 or 95 per cent N_2 and 5 per cent CO_2, although occasionally stimulation was noted with the employment of 95 per cent O_2 and 5 per cent CO_2. When using a Krebs-Ringer phosphate medium with 0.5 to 2.0 per cent bovine albumin, Lynn et al. consistently observed stimulation of glucose uptake by epinephrine and ACTH. In this medium triose isomerase activity was inhibited and the fate of C-1 and C-6 from glucose-C^{14} differed somewhat from that observed when a bicarbonate medium was used. However (and of importance with respect to the argument as to whether the action of these hormones on triglyceride hydrolysis is primary or secondary), irrespective of whether net glucose uptake was stimulated or inhibited, fatty acid accumulation in the tissue and/or the medium always occurred in response to ACTH and epinephrine.

The studies of Lynn and Cahill suggest that the increase in glucose uptake by adipose tissue stimulated by epinephrine or ACTH is secondary to the hydrolysis of triglycerides and that the latter may represent the primary site of action of the hormones. The glycerol is lost to the medium, and is poorly utilizable for re-esterification. Instead, as is apparent from the heavy labeling of the glyceride-glycerol with C^{14}, medium glucose is utilized for the formation of glycerol-phosphate, which is used for re-esterification. Simply increasing the content of albumin-palmitate[18] or of butyric acid[20] in the medium accelerates glucose uptake.

On the other hand, there are data which suggest that under certain circumstances excessive and/or persistent accumulation of free fatty acid within the adipose tissue may eventually lead to an inhibition of glucose uptake, perhaps by altering the pH or physical properties of the cytoplasm. Table II describes an experiment consistent with this interpretation. Samples of adipose tissue were removed from fasted rats and incubated for thirty minutes in a Krebs-Ringer bicarbonate medium containing no albumin (O) or 4 per cent bovine albumin (A). Ten μg. of epinephrine were added to each flask except the appropriate control vessels. At the end of the incubation the tissues were removed, washed and then transferred to new flasks containing the same medium (with or without albumin), and incubated for two and a half hours; this time no hormone was added. An inverse correlation was found between the utilization of glucose and the final concentration of free fatty acid in the tissue. The lowest glucose uptakes and the highest fatty acid concentrations were in those tissues which were exposed the longest to a medium without albumin (OO and AO), while the reverse was found in the tissues from which fatty acids could best escape into the medium by combining with albumin (AA and OA). Lynn obtained similar results

showing that glucose utilization, fatty acid release and oxygen consumption of epinephrine-stimulated tissue fall off with time during incubation in a low albumin medium, while glycerol continues to pour out unchecked. In other experiments he observed that although epinephrine and 0.2 μM butyric acid separately stimulated uptake of glucose by adipose tissue, together they inhibited it, presumably because of excessive accumulation of fatty acid.

A working hypothesis is that glucose utilization is stimulated *in vitro* as long as exchange of fatty acid between triglyceride in the cell and albumin in the medium is rapid enough to prevent excessive accumulation of free fatty acid in the tissue. The conditions for accelerated glucose uptake may not be met when exchange is limited either by absence of albumin or by a pre-existing high concentration of fatty acid in the medium as when the tissue is stimulated by a lipolytic hormone. It is unlikely that these circumstances ever exist *in vivo* with adequate circulation. Hence the impaired glucose uptake in our experiments must be considered unphysiologic. Although increased glucose uptake would appear to be the physiologic response to epinephrine *in vivo*, this remains to be demonstrated. With an intact circulation, fatty acids may be swept out of the adipose tissue so rapidly that they would not serve as the same stimulus for glucose uptake and re-esterification as they do in the *in vitro* system. If epinephrine and norepinephrine do, in fact, have an important physiologic role in fatty acid mobilization from adipose tissue, then the reverse process of fatty acid re-esterification and associated glucose uptake would be expected to be of minor importance, quantitatively. Further investigation is obviously needed before the *in vitro* data can be confidently interpreted in physiologic terms.

SUMMARY

The role of hormones in mobilization of free fatty acids from adipose tissue is reviewed.

Evidence is discussed indicating that epinephrine, norepinephrine, ACTH and other hormones which stimulate release of fatty acids from adipose tissue, do so by promoting lipolysis. Their actions in either promoting or inhibiting glucose uptake *in vitro* are interpreted as being secondary to changes in intracellular concentration of free fatty acids.

The action of insulin in retarding net free fatty acid release is mediated through a primary action on carbohydrate metabolism and not by directly inhibiting lipolysis.

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DISCUSSION

DR. VINCENT P. DOLE (*New York, New York*): As you know, many people are interested in the difference between stable diabetes and brittle diabetes. The notion that growth hormone might promote an excess release of fatty acid from the depots in patients with brittle diabetes and lead to ketosis and loss of weight, and so on, has been discussed.

Would you comment on the possibility that ACTH might be a factor in all of this?

DR. WHITE: The problem with ACTH, as it exists now, is that an extra-adrenal activity in the intact human being has not yet been demonstrated. We are certainly not willing to speculate further on this problem at this time.

The situation with growth hormone is really very interesting. The main conclusion, after our many different attempts with this system to demonstrate lipolytic activity, is that it probably does have such activity. In view of the fact that the activities of growth hormone and ACTH, *in vivo*, are so similar, it is surprising that growth hormone, if it acts directly on the tissue, is not more active *in vitro*. We wonder, therefore, whether it does not have more than one action in stimulating release of fat from adipose tissue in the intact animal.

DR. DOLE: Dr. Raben, do you not have a defense of growth hormone?

DR. RABEN (*Needham, Massachusetts*): I have no defense at all for the poor performance of growth hormone in the *in vitro* system. From my own experience it takes about 10 μg. of human growth hormone with rat adipose tissue to get an effect *in vitro*.

Of course, as Dr. White has indicated, this would only have to have one part in ten thousands contamination with ACTH to get the effect from ACTH and possibly other contaminants.

On the other hand, in the intact animal, the effect is most impressive, that is, in terms of its effect on plasma fatty acids and also on the behavior of the epididymal fat removed from treated animals. I think, indeed, that the effect on plasma unesterified fatty acids can be obtained with the smallest amount of growth hormone ever shown to have a biologic effect. Also, the effect is one of the quickest of all the demonstrated effects of growth hormone. When a hypophysectomized rat is given 1 μg. of growth hormone a day for four days (approximately a fifth to a tenth of the mininal growth-promoting dose) and the adipose tissue is removed eighteen hours after the last injection, the adipose tissue *in vitro* puts out fatty acids in an *in vitro* system; whereas the adipose tissue from non-treated hypophysectomized rats does not.

When ACTH is administered that much beforehand, that is, that long before taking out the fat, there is no such effect. But, of course, that is not a fair test for ACTH, since it is a very short-lived hormone.

Incidentally, when I say that the fat from the hypophysectomized rat puts out no fatty acid *in vitro*, this applies to fat from most animals. There is something strange about the behavior of fat *in vitro*, either because the *in vitro* medium is not ideal or else it has been removed from some influence that is only present in the body or is ephemeral, transient. Fat from fasted animals puts out practically no fatty acids during incubation.

I know this is in disagreement with published work, but the reason for this is that most of the published work has not measured the amount of fatty acid present in the tissue at the time it is removed. If one titrates the tissue at the time of removal, one finds that there is more fatty acid in the tissue from fasted rats than in tissue from fed rats. During the period of incubation, very little is put out by either tissue, although a little bit more is put out by the fasted animal. However, if glucose and insulin are added in such incubations, there is actually a disappearance of fatty acid in the esterification, as can be demonstrated. If you put large amounts of fatty acid into the medium, you can actually cause much esterification.

We are getting away from the question of growth hormone's poor performance *in vitro*. I think it makes suspect, indeed, all the demonstrations of *in vitro* effects of growth hormone, because of the large amounts required.

DR. WHITE: We have had occasion to confirm your observations with animals fasted up to nine days and note, too, that their output of fatty acids, if you excise the tissue and incubate it in a variety of different media, is much less than you would expect.

Our own view is that fat mobilization from adipose tissue, in the form of NEFA, is not secondary to lack of glucose but due primarily to hormonal stimulation of the tissue. In excising the tissue, you remove this source of stimulation.

We do not have any further data than those presented to support our conclusion.

DR. R. H. WILLIAMS (*Seattle, Washington*): I would like to ask Dr. Dole or Dr. White why it might not be possible for the adrenal to play a more important role than either the growth hormone or the ACTH, particularly in view of Scow's work, and in view of the fact that with adrenalectomy, the patients are so much less inclined to have diabetic acidosis even though gluconeogenesis has been decreased tremendously.

DR. DOLE: At the present moment we are gathering a great many detached facts and are really not able to give good explanations of clinical syndromes. I think that everybody who is interested in it will be able to raise one or another possibility.

DR. WHITE: I cannot add anything except that Dr. Scow's results are very puzzling to me, and Dr. Engel believes that cortisone is antiketogenic only when insulin is present and hence there may not be any real contradiction between his work and Dr. Scow's.

DR. DWIGHT J. INGLE (*Chicago, Ill.*): There have been a number of studies performed since 1941 which have confirmed Skow's observation that if you give ACTH or adrenal steroids to a depancreatized animal, it will induce ketosis. I think the first studies were done by Thorn and me. There have been numerous other studies in which we were able to induce severe ketosis in partially depancreatized and even in normal animals. However, in all these situations the ketosis co-existed with a glycosuria so severe that had it been produced by any other means, there would also have been ketosis.

DR. EDGAR S. GORDON (*Madison, Wisconsin*): Dr. Knobile at Harvard has told me of his experiments using a system, I think, very much like yours, in which he has demonstrated a remarkable release of NEFA as a result of incubation with growth hormone. There is a release, not only of NEFA, but also of triglycerides. I do not know the details of the buffer system used or the details of this technic.

We have administered an intravenous infusion of ACTH to a bilaterally adrenalectomized human subject, and we noted two interesting things. One was quite a remarkable rise in blood cholesterol levels during the infusion: whereas you will recall that in the human subject with the adrenal glands intact, there is usually a suppression or a depression. We also noted a considerable rise in the NEFA levels during this infusion. This would appear to be the experiment you were looking for in the intact human being.

Has any work been done with fat sources other than the epididymal pad? Is there any priority in the fat depots that are mobilized by this means in a normal animal?

DR. WHITE: Fat from a number of different sources has the same reaction as the epididymal pad. It is simply a convenient source. Perirenal and subcutaneous fat has been used once or twice to be sure that this assumption was correct. Dr. Lynn, at Duke University has performed a number of studies with other metabolic activities and found it true of fat from various sources in the rabbit and rat. It is not peculiar to the epididymal fat.

Factors Affecting Fat Mobilization from Adipose Tissue

ERNST WERTHEIMER, M.D.* MARGIT HAMOSH, M.SC.† AND ELEAZAR SHAFRIR, M.D.‡

MANY experiments on adipose tissue have been performed, using randomly selected fat bodies. Apparently there is scant knowledge as to which fat bodies can be used in order to compare the metabolic characteristics of various adipose tissues. In our studies, we have been concerned with to what extent a given adipose tissue is representative of the general behavior of stores of body fat, or whether there is a tissue specificity for certain responses. The results may be seen in Figures 1 through 3.

In Figure 1, four different tissues, removed from rats fasted one day, were compared in order to show the amount of unesterified fatty acids (UFA) released when incubated in 5 per cent albumin solution with or without stimulation by epinephrine. It should be noted that all tissues tested are highly susceptible to epinephrine *in vitro*, but the extent of stimulation by epinephrine seems to be highest in the experiment with epididymal fat pad. On the other hand, the initial release of UFA was larger in the mesenteric tissue.

No explanation can be offered for these differences beyond some speculation on the possibility of denser innervation in the mesentery, which may keep it under more rigid autonomous control. This tissue appears to be more dynamic in lipid movement, as observations revealed that it is first affected in tendencies toward adiposity and leanness.

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Presented at the Brook Lodge Invitational Symposium on Energy Balance, sponsored by The Upjohn Company, September 29, 1959, at Brook Lodge, Augusta, Michigan.

Figure 2 shows that the rate of UFA release correlates well with glucose uptake in a given tissue, as exemplified by the values obtained in the mesenteric and epididymal tissues. Figure 3 depicts the response of various tissues to the treatment of the donor animal with insulin. The brown interscapular fat responds most rapidly and to a larger extent with glycogen deposition, but this activity does not persist and declines steadily. On the other hand, the *in vitro* glucose uptake of the epididymal fat body has been found extremely sensitive to the presence of insulin, enabling the assay of minute quantities of the hormone.[1]

In other preliminary experiments some species have shown differences in the *in vitro* tissue UFA release and its response to epinephrine. The rabbit seems to differ from omnivorous animals by having the lowest tissue UFA activity. We found[3] that the herbivora (guinea pigs and rabbits), following fasting and recovery feeding, showed an irregular and extended course of glycogen deposition coupled with low fat increment, which rose to appreciable levels only one week after refeeding.[2] These observations are included to illustrate that among the species there may be wide differences in the relative contribution of carbohydrate and fat metabolisms to caloric homeostasis. This is particularly true in the rabbit, which usually has higher stores of glycogen than the omnivorous animals, and may depend less on UFA mobilization.

As shown by several groups of investigators, epinephrine has been found to be one of the most potent factors eliciting increased UFA release. Our experiments have indicated that in many physiologic and pathologic conditions, the increased UFA mobilization may be effected through the mediation of sympathetic dis-

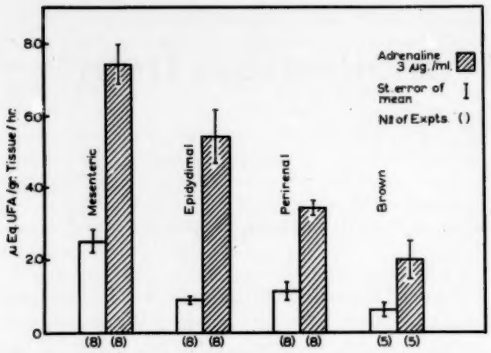

FIG. 1. Release of unesterified fatty acid from different rat adipose tissues *in vitro*.[3] Tissue pads, 200 to 300 mg. in weight, from rats fasted for twenty-four hours, were incubated in air for twenty-four hours in 2 ml. of 5 per cent albumin solution at 37°c., with or without 3 µg. per ml. epinephrine.

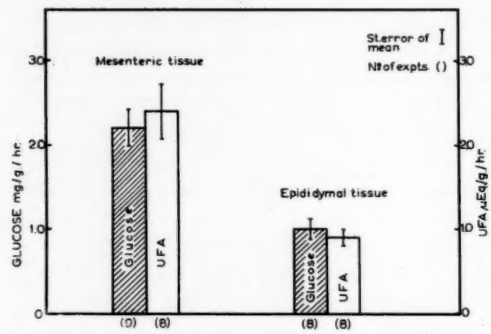

FIG. 2. Uptake of glucose and release of UFA from rat mesenteric and epididymal tissues *in vitro*.[3] The release of UFA was measured as described in Figure 1. Uptake of glucose was measured during incubation in homologous serum of 120 to 150 mg. per 100 ml. of glucose content.

charge. In Figure 4, it may be seen that the release of UFA from mesenteric adipose tissues *in vitro* (taken from animals in hunger states, after exposure to low temperatures, or after treatment with triiodothyronine, alloxan or endotoxin) may be effectively prevented by pretreatment with Dibenzyline®.[3]

The effect of exposure to cold is of interest, because contrary to previous concepts, the adipose tissue responds almost instantaneously to the increased energy requirement of the organism (Fig. 5). It is indeed striking that not only in caloric adjustment of hunger but

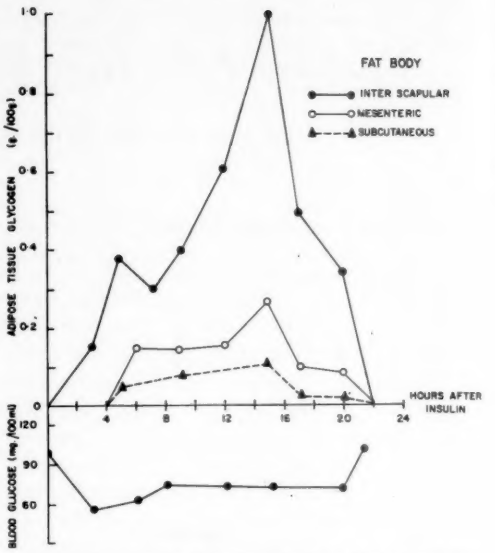

FIG. 3. Time course of hypoglycemia and glycogen deposition in different adipose tissues of rats after treatment with insulin (1 unit per 100 gm.).

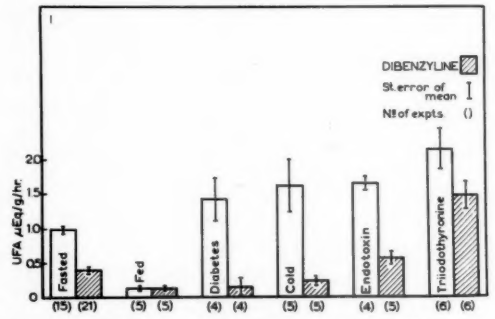

FIG. 4. Effect of pretreatment with Dibenzyline on *in vitro* relase of UFA from mesenteric tissues of rats at different conditions of increased fat mobilization.[3] Five mg. of Dibenzyline was implanted subcutaneously forty-eight hours before the rats were killed. Release of UFA was measured after two hours incubation of the tissue in 2 ml. of 5 per cent albumin solution in air at 37°c. Effect of fasting was obtained after forty-eight hours of food deprivation. Diabetes in fed rats was produced by one subcutaneous injection of 15 mg. per 100 gm. of alloxan in citrate buffer pH 4, ninety-six hours before the rats were killed. Fed rats were exposed to 4°c. for two hours. Serratia marcescens endotoxin, 0.5 mg. per 100 gm., was injected intraperitoneally into animals fasted for twenty-four hours. Ten minutes later, the animals were killed. Triiodothyronine was injected three times into fed rats in doses of 0.25 mg. per 100 gm. at sixteen hour intervals, and the animals were killed forty-eight hours after the last injection.

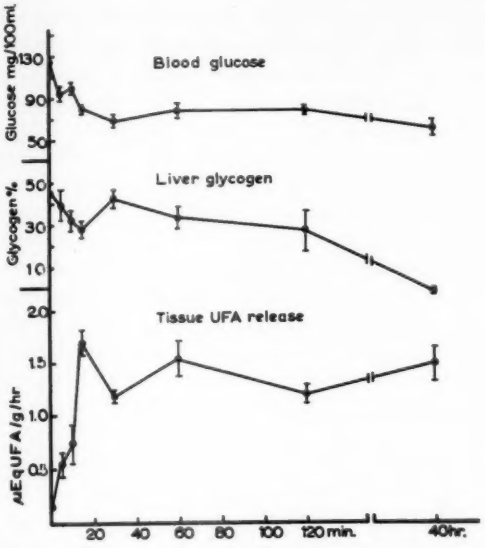

FIG. 5. Effect of low temperature on release of UFA of rat tissue, blood glucose levels and liver glycogen content.[3] Groups of fed rats were killed after exposure, for different periods of time, to temperature of 4°C., UFA release *in vitro* was measured as described in Figure 1.

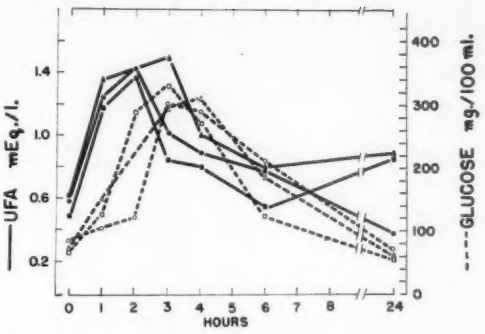

FIG. 7. Time relationship between plasma UFA and blood glucose elevations in dogs receiving subcutaneous injections of 0.6 mg. per kg. epinephrine oil.[4]

also in chemical thermoregulation, the stores of fat along with stores of carbohydrate fulfill the immediate demands for energy.

As shown in Figure 6, administration of endotoxin contributes to the elucidation of the general mechanism of action of toxic substances. The responses of plasma UFA and glucose after the injection of endotoxin are similar to those occurring after the injection of epinephrine (Fig. 7). Their inhibition, by pretreatment with autonomic blocking agents, lends further support to the thesis that one of the primary effects of the toxins is provocation of powerful sympathico-medullary hyperactivity, resulting in the mobilization of UFA in the tissues and glycogenolysis.

In diabetic rats, mobilization of UFA, after treatment with Dibenzyline, is particularly

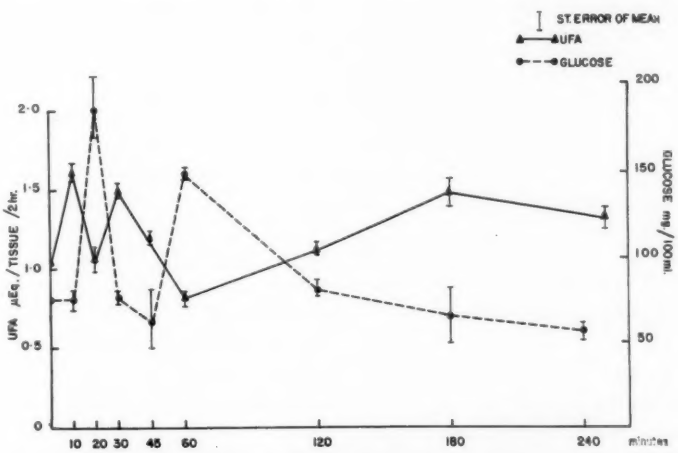

FIG. 6. Time course of levels of blood glucose and release of tissue UFA after injection of bacterial endotoxin.[3] Groups of animals were killed at different times after the injection of 0.5 mg. per 100 gm. of serratia marcescens endotoxin. Release of UFA *in vitro* was measured as described in Figure 1.

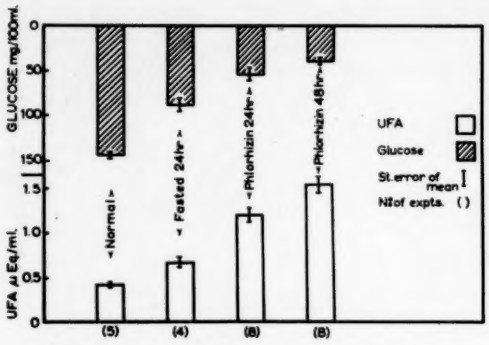

FIG. 8. Levels of blood glucose and plasma UFA in rats at different stages of carbohydrate depletion.[1] Twenty mg. phlorhizin in 0.1 per cent sodium bicarbonate solution per 100 gm. body weight were administered once (twenty-four hour treatment) or three times at sixteen hour intervals (forty-eight hour treatment).

reduced (Fig. 1). This may be due to the fact that the mobilization induced by endogenous epinephrine is more pronounced in animals with diabetes because of lack of adequate antagonizing influence of insulin in the direction of fat accumulation. The interplay of the hormones, insulin and epinephrine, appears to be crucial in determining the extent and the pattern of the metabolism of adipose tissue and, by the same token, in regulating the energy metabolism of the body as a whole. Their opposite effects are exerted directly on the adipose tissue, which seems to be the principal target of their action. These hormones are active *in vitro* in trace amounts and have immediate physiologic effects on the tissue when administered *in vivo*.

With respect to triiodothyronine (Fig. 1), the blocking agent had little effect on tissue UFA release. This may suggest perhaps that this hormone has a direct effect on the tissue. A direct action was postulated on the basis of recent observations that UFA elevation is one of the earliest symptoms of induced thyrotoxicosis in patients.[4]

The various factors which (through the mobilization of UFA) affect the caloric balance, do so by direct impact on the cellular metabolism of the adipose tissue. Many observations show that the underlying mechanism of their action appears to involve specific alterations in the utilization of carbohydrate within the tissue. Such changes in the metabolism of adipose tissue may be caused by different levels of glucose in the perfusing blood. A reciprocal relationship appears to exist between plasma glucose and UFA levels at different stages of carbohydrate depletion (Fig. 8). The release of UFA from tissues stimulated by epinephrine may be suppressed by glucose in both *in vivo* and *in vitro* experiments.[5,6] On the other hand, it does not seem probable that the tissue will regulate its UFA output following changes of glucose concentration alone. Our experiments show that an increase in the UFA mobilization of fed rat adipose tissue, incubated in homologous serum, cannot be induced by merely lowering the glucose content of the serum. Conversely, the UFA output from starved rat tissue cannot be reduced by raising the concentration of glucose in the corresponding serum.[7] The UFA release may be, however, very effectively reduced by glucose and insulin.[8]

This point may be further illustrated by several situations in which the mobilization of UFA is independent of glucose levels in the circulating blood. The outflow of UFA from tissue is reduced either in hypoglycemia after administration of tolbutamide,[9] or in hyperglycemia after administration of glucagon.[10,11] It is increased in cases of hypoglycemia from phlorhizination or hyperglycemia from diabetes. The common finding in all cases associated with high levels of UFA is the abolished arteriovenous difference in glucose concentration. This suggests the conclusion that it is the rate of cellular carbohydrate utilization that is instrumental in caloric balance. This is a prominent example of the regulatory function of a tissue exerted through its own metabolism.

A somewhat speculative scheme of metabolic interrelations in the adipose tissue which may help to clarify the mechanism by which the impairment in glucose metabolism causes an increased release of UFA is shown in Figure 9.

The hypothesis has been advanced, that when glucose is available its oxidative breakdown supplies an essential factor for the building up of the fatty acid chain in the form of reduced triphosphopyridine nucleotide (TPNH), while its glycolytic catabolism provides the acetyl coenzyme A complex for the elaboration

of the primary building stones.[12,13] When the metabolism of glucose is deficient, fatty acid elaboration ceases and the quantity of stored fat is reduced. Such findings would not support the concept that the commencing outflow of UFA originates from synthesis *de novo*.

Attention should be drawn, to the fact that during the metabolism of glucose another metabolite is evolved, which is requisite for the containment of the newly synthesized fatty acids within the tissue. This metabolite is the glycerol moiety of stored fat (EFA), derived from an "active" precursor, and capable of entering into the esterification reaction with the acyl-CoA residues (FA-CoA). The nature of the "active" glycerol compound is not yet ascertained though evidence points to α-glycerol phosphate in analogy with findings in the liver esterifying systems.[14]

A dynamic equilibrium appears to exist between the stored glyceride esters (EFA) retained in the droplets of fat of the adipose tissue cells and the intrinsic UFA pool of the tissue.[15] The hydrolysis is probably effected through a lipase of poorly defined characteristics, while the re-esterification of the freed carboxylic groups requires an ATP-CoA activating system.[16,17] The esterification reaction is enhanced by glucose and insulin as shown by the diminution of pools of tissue UFA in their presence.[18,19] However, this pool is increased in hunger, diabetes[19] and during stimulation by epinephrine or corticotropin.[20,21]

There is the possibility that the causative factor for the increased production of UFA in absence of proper glucose utilization is a disturbance in the re-esterification reaction. Such disturbance may be due either to the limited supply of "active glycerol" and/or to the interference of the ATP-CoA dependent activation of newly hydrolyzed fatty acids. Cleavage of glyceride ester bonds would produce a "non-active" glycerol which, due to the possible absence of a specific kinase in adipose tissue,[14] may be unable to enter into the reaction.

Furthermore, the liberated glycerol may be quickly metabolized, especially when there is a scarcity of glucose. With the equilibrium disturbed and UFA concentration rising, it is possible that a secondary activation of lipase takes place. An activation of certain tissue lipases by the product of their hydrolysis has been reported[22,23] and during the study of conditions for *in vitro* UFA release, higher yields of tissue UFA were obtained at an optimal initial concentration of unesterified fatty acids rather than with UFA-poor albumin.[24] Since epinephrine does not directly enhance tissue lipase[25] despite its marked effect on UFA production, it is difficult to avoid the conclusion that the increase in lipolysis of stored glycerides is effected indirectly by imposing a limitation on esterification of fatty acids, which is linked to the metabolism of glucose. Glucagon and corticotropin at higher concentrations appear to act similarly to epinephrine. This is indicated by stimulation of tissue phosphorylase activity and increased glycogenolysis in the presence of the above hormones.[26] A shift in the pathway of glucose metabolism was observed when adipose tissue was incubated with such a potent UFA mobilizer as growth hormone.[27] Thus, the possibility that the release of stored fatty acid depends on a certain route of glucose catabolism just as fat elaboration depends on another, should be borne in mind.

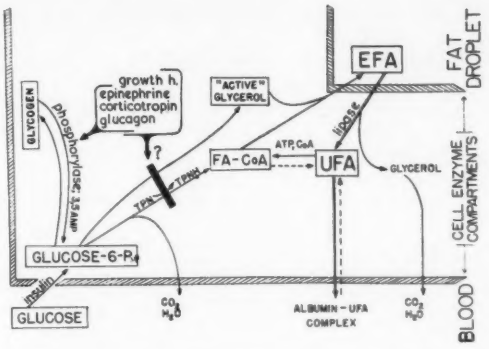

FIG. 9. Schematic representation of possible mechanism leading to increased UFA output from adipose tissue. It is postulated that the hormones indicated in the figure interfere with the re-esterification reaction of newly hydrolyzed tissue UFA. (See text.)

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DISCUSSION

DR. VINCENT P. DOLE (*New York, New York*): It has been suggested that triglycerides might be discharged from adipose tissue. There has been much discussion about UFA and one fears the overemphasis of a single component in a complicated system. Do you think that it is possible that our experimental technic has just not been favorable for demonstrat ng a release of triglycerides, or do you have reason to think that this is the sole means of mobilization?

DR. WERTHEIMER: Some years ago it was thought that triglycerides were the main substance. How do you think they are transported from adipose tissue to other tissues to the liver? I see no possibility of the transport of triglycerides from the adipose tissue to the liver or to other organs, but it may be possible.

DR. RABEN (*Needham, Massachusetts*): Dr. Wertheimer has mentioned the difference in behavior of fat from several areas, and I would like to mention a difference in one other type of fat; human subcutaneous fat. Human subcutaneous fat, when incubated *in vitro*, will respond to epinephrine with an output of fatty acids. This output is very small compared to that of the rat, but it is a regular effect. On the other hand, we can never obtain an effect with ACTH or with human growth hormone.

DR. WERTHEIMER: In the different metabolism of adipose tissue, I see one opportunity to explain the localized deposit. I cannot explain a local deposit without accepting any kind of special metabolism of special adipose tissue.

DR. RICHARD HAVEL (*San Francisco, California*): A few years ago we sought to investigate the effect of the sympathetic nervous system on the mobilization of fat from adipose tissue, using the newer technics and in view of our knowledge of UFA. We have performed some experiments similar to what Dr. Wertheimer has reported here, using a different species (dog) and using a slightly different approach.

It occurred to us that if the sympathetic nervous system is important in this process, we now know that the hormone liberated from the sympathetic nerve endings, except in the adrenal medulla, is norepinephrine, not epinephrine. So we tested the effect of norepinephrine injection upon plasma UFA concentration and found it was very potent, just as potent as epinephrine, even though it has, as you know, very little effect upon raising blood sugar levels.

We then reasoned, much as Dr. Wertheimer has, that if the sympathetic nervous system is causing mobilization, then blocking its activity should decrease mobilization. We have administered ganglionic blocking agents—as opposed to the adrenergic blocking agent, (Dibenzyline®, which he employed) to human subjects and dogs, blocking sympathetic activity by blocking the transmission of impulses across the ganglia. We have found a rapid fall in concentration of UFA with the administration of hexamethonium, for example, in dogs, just as rapid, just as profound as that observed after administration of insulin or glucose, *in vivo*.

Also in dogs, we have administered Dibenamine®, an adrenergic blocking agent, and found that this blocked the effect of both epinephrine and norepinephrine upon the release of fatty acids, as measured by an increase of fatty acid concentration in the plasma. So I think all these things simply support what Dr. Wertheimer has reported. And in further support of

his statement about triglyceride release, in answer to Dr. Dole, we have found no increase in triglyceride concentration, acutely, after epinephrine or norepinephrine, when UFA concentrations rise, say, three- or four-fold.

DR. WERTHEIMER: I think it may be possible that norepinephrine is more important than epinephrine, because with norepinephrine there is a long duration of UFA release. With epinephrine, the release is of short duration, because there is hyperglycemia and with hyperglycemia there is a decrease of UFA release.

DR. T. M. CHALMERS (*London, England*): Dr. Havel, would you make it clear whether you observed the same decrease of UFA after administration of Dibenamine as you did after administering ganglion blocking agents?

DR. HAVEL: Dibenamine is a very complicated drug. When a unit is administered, there is an acute release of epinephrine and norepinephrine from the adrenal medulla and a concomitant rise in blood sugar and blood UFA, and there is also a marked central excitatory state. This lasts an hour or so, and thereafter there is an adrenergic blockage, which persists for several days. During the period of this blockage, UFA concentrations are at normal levels, but the rise produced by injected catechol amines is prevented.

This is in keeping with other concepts that the blockade of injected amines by adrenergic blocking agents is much more complete than that of transmitted sympathetic impulses. We have not sympathectomized them by this. We have simply prevented the effects of material released from the adrenal modulla.

I think this reinforces the concept that epinephrine is not too important here. This is released intermittently with stress. But there is probably a constant release, I think, of norepinephrine, which is important, and this can be blocked with hexamethonium.

DR. KENNETH CRISPELL (*New York, New York*): Dr. Wertheimer, were your studies with triiodothyronine on UFA levels performed in the intact animal?

DR. WERTHEIMER: That was not done in our laboratory.

DR. CRISPELL: Was that thyroxin or triiodothyronine?

DR. WERTHEIMER: That was in the human being.

DR. CRISPELL: Did you say you believed it was a direct effect on NEFA levels and not through epinephrine?

DR. WERTHEIMER: It must be a direct effect, because we note so little effect of the Dibenzyline. And, on the other hand, after five hours there is the first effect of triiodothyronine. We observe the effect before the metabolism is influenced. It is one of the first effects of triiodothyronine.

DR. CRISPELL: Is that before a rise in basal metabolic rate?

DR. WERTHEIMER: Yes.

DR. CRISPELL: Do any of the other agents which cause a rise in basal metabolic rate cause elevation of the UFA levels, such as dinitrophenol or salicylates?

DR. WERTHEIMER: I do not know.

DR. CRISPELL: Dr. Hammond, who now works with us, who worked with Dr. Hickler at the Peter Bent Brigham Hospital, has an interesting study of a patient with autonomic insufficiency who has postural hypotension. With a standard tilt procedure they can demonstrate that the normal person releases norepinephrine or catechol amines and also releases NEFA. This patient with autonomic insufficiency has no release of norepinephrine and also has no release of NEFA into the plasma.

DR. ESTELLE R. RAMEY (*Washington, D. C.*): We have studied the epididymal fat body *in vivo* with blocking agents during the fasting condition. We were unable, with Dibenzyline or hexamethonium, to obtain significant effects on the changes in total fat content, a very crude measurement, within the epididymal fat body in a twenty-four hour fast. When we blocked with ergotamine, we were able to demonstrate that during the fasting period, the epididymal fat body lost less of its total fat than the normal unblocked animal does during this fasting period. This was, I think, published in *Proceedings of the Society for Experimental Biology and Medicine.*

I do not know of anybody else who has been interested in the epididymal fat body *in vivo*, with these blocking agents, and I wonder whether you have any experience with this.

DR. WERTHEIMER: These experiments have been started but are not yet complete. I think we must now begin with the pharmacology of adipose tissue and see if substances from these blocking agents accumulate, especially in adipose tissue.

It will also be interesting to discover if there are substances—and there are such substances among barbiturates, for instance—which accumulate especially in adipose tissue, producing a local necrosis in adipose tissue without general necrosis. That is part of the program we are working on. I would like to ask you what blocking substance you have used.

DR. RAMEY: We used ergotamine tartrate, Dibenzyline, hexamethonium and reserpine, as a central blocking agent, and we obtained no effect on inhibition of movement of fat out of the epididymal fat depot during a fast except with ergotamine tartrate. The other blocking agents, in our experience, were ineffective.

DR. WERTHEIMER: How long have you waited after the injection?

DR. RAMEY: We used each of these blocking agents in two ways. In one set of experiments we injected the material just prior to the onset of fasting. In another set of experiments, we pre-treated for two days with the blocking agent and then administered some blocking agent once during the twenty-four hour fast as well.

Again, I will repeat that only the ergotamine elicited significant results on this very crude system of using the animal as his own control, and measuring total fat content of one epididymal fat body after a fast as compared to the total fat content on the other side before the fast.

The Obese Hyperglycemic Syndrome of Mice as an Example of "Metabolic" Obesity

JEAN MAYER, PH.D., D.SC.*

THE ASSIGNED subject of this report is the obese hyperglycemic syndrome and its genetic, metabolic and physiologic effects. To give a comprehensive review of this interesting syndrome would entail several chapters, considering the amount of information accumulated. It therefore, appeared to be more in keeping with the general character of this symposium to use this syndrome simply as a typical illustration of the concept of "metabolic obesity" as opposed to "regulatory obesity."

I have been asked on numerous occasions for a comprehensive list of research papers on studies of the obese hyperglycemic syndrome carried out in our laboratory; this list is presented separately as a bibliography, which also incorporates some of the more significant reports of studies made elsewhere on the subject. The references given in the text are for general references on the subject of obesity and are obviously by no means exhaustive nor even perhaps entirely representative. No effort has been made to document every statement in the references.

The development of the various forms of obesity can be considered from either the point of view of etiology or of pathogenesis. The etiologic approach has been developed at length in a previous review[1] in which genetic, traumatic and environmental factors were distinghished (Fig. 1). Obviously, such a distinction, although useful, necessitates a certain degree of oversimplification; in order for obesity to develop, there has to be permissive interaction of genetic and of environmental factors, or of traumatic factors with genetic and environmental background. However, this simplification provides a useful classification for singling out the characteristic element in the etiology. Table 1 is an adaptation of a table given in a more extensive review[2] which includes references for the different types cited.

My associates and I have also grouped obesities into two categories, these being regulatory and metabolic. We found that a general distinction could be made between regulatory obesities, in which the primary impairment is of the central mechanism regulating food intake, and metabolic obesities, in which the primary lesion is an inborn or acquired error in the metabolism of tissues, *per se*. In the first case, habitual hyperphagia may lead to secondary metabolic abnormalities. In the second case, peripheral metabolic dysfunction may in turn interfere with the proper function of the central nervous system. This difference has been demonstrated in our laboratory by comparisons between different types of obesity in mice.

Regulatory obesities are exemplified by the hypothalamic obesities induced either by surgical (stereotaxic) bilateral destructions in the ventromedial nuclei[3] or by extensive symmetrical destructions in the ventromedial area after administration of goldthioglucose.[4] Mice with these syndromes show hyperphagia and may gain up to four times their normal weights. Their rate of lipogenesis and cholesterogenesis (as measured by incorporation of radioacetate into hepatic and extrahepatic fatty acids and cholesterol, *in vivo* and *in vitro*) increases in proportion to the amount they are allowed to overeat. Fasting brings lipogenesis down to normal fasted levels. Their

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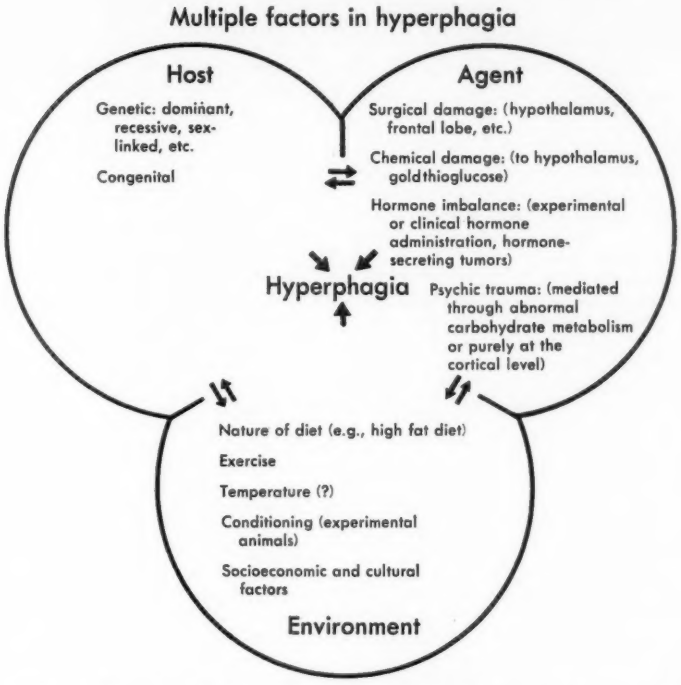

FIG. 1. A schematic view of constitutional (genetic and congenital), traumatic and environmental factors in the etiology of obesity. (From: MAYER, J. Etiology and pathogenesis of obesity.) *Postgrad. Med.*, 25: 631, 1959.

rate of absorption of glucose by the intestine increases, but this appears to be a secondary result of hyperphagia. When such obese animals are reduced by fasting, their body composition returns to normal as their weight returns to normal. Animals made hyperphagic by conditioning are also examples of regulatory obesity.

The situation in metabolic obesities such as the hereditary obese hyperglycemic syndrome or obesity due to grafting of ACTH-secreting pituitary tumors is in striking contrast to that in regulatory obesities (Table II).

The obesity of mice with the hereditary obese hyperglycemic syndrome is as extreme as that observed in mice of the hypothalamic types. However, their hyperphagia is usually less pronounced than the latter's, since their caloric surplus is partially due to their relative inactivity. In the first group of mice, either marked hyperglycemia already exists or it is readily elicited by the administration of growth hormone; whereas the hormone has little or no effect on the levels of blood glucose in normal littermates or in littermates made obese by hypothalamic lesions induced by stereotaxis or by administration of goldthioglucose. Mice with the obese hyperglycemic syndrome show marked hypercholesterolemia. In addition, they evince a variety of atypical responses to the administration of hormones. Although their means of "physical" defense against cold (such as pilo-erection and vasoconstriction) are intact, the animals are incapable of raising their metabolism when exposed to low temperatures and therefore die rapidly.

Mice with the obese hyperglycemic syndrome show considerably hypertrophied islets of Langerhans with increased numbers of both alpha and beta cells, increased pancreatic insulin and glucagon content and increased circulating insulin. The most recent findings concerning hormone concentrations support a previously postulated etiology of primary pancre-

TABLE I
Types of Obesity

In Mice

Genetic:
"Yellow" obesity, associated with coat color: heterozygous, dominant character; normal mating. "Hereditary obese hyperglycemic syndrome"; homozygous, recessive character associated with absence of mating. "NZO" obesity; homozygous recessive character, normal mating.
Of hypothalamic origin:
Spontaneous; surgically induced; induced by goldthioglucose.
Of endocrine origin:
Caused by grafting of pituitary tumors secreting adrenocorticotrophic hormone.
Otherwise induced:
By high-fat diet.

In Rats

Genetic:
Associated with diabetes.
Of hypothalamic origin:
Induced by bilateral or unilateral lesions.
Of other central nervous system origin:
From frontal lobe damage.
Of endocrine origin:
From hypertrophy of adrenal cortical tissue; from prolonged treatment with protamine zinc insulin, or insulin with forced feeding; after thyroidectomy with hypothalamic lesions or with forced feeding.
Otherwise induced:
By immobilization; by high-fat diet; by conditioning.

In Dogs

Genetic:
In the Shetland sheepdog, recessive character.
Of hypothalamic origin:
Spontaneous; surgically induced; due to paraventricular degeneration caused by corticotrophin or cortisone.
Otherwise induced:
By immobilization

In Monkeys

Of hypothalamic origin:
Surgically induced.
Of other central nervous system origin:
Surgically induced by lesions of the thalamus.

In Farm Animals

Genetic:
In strains selectively bred for fat, in particular, pigs bred for lard.
Of endocrine origin:
Induced by castration and by estrogens in the fowl; by castration and implants of estrogens in male cattle.
Otherwise induced:
By immobilization in pigs, cattle and geese; by forced feeding in geese for production of foie gras.

In Man

Genetic:
A multiplicity of genes have been studied by Newman, von Vershuer, Bauer, Gurney, Rony, Angel and others; in congenital adipose macrosomia; in monstrous infantile obesity; associated with Laurence Moon Biedl syndrome; associated with hyperostosis frontalis interna; associated with von Gierke's disease; in familial hypoglycemosis (congenital lack of alpha cells).
Of hypothalamic origin:
In dystrophia adiposogenitalis, with discrete or diffuse hypothalamic injury; occasionally with panhypopituitarism and narcolepsy; Kleine Levin syndrome.
Of other central nervous system origin:
After frontal lobotomy; in association with cortical lesions, in particular, bilateral frontal lesions
Of endocrine origin:
With insulin-producing adenoma of the islets of Langerhans, with diffuse hyperplasia of the islets, and in association with diabetes; with chromophobe adenoma of the pituitary without hypothalamic injury; in Cushing's syndrome (hyperglycocorticoidism); from treatment with cortisone or adrenocorticotropic hormone; in the Bongiovanni Eisenmenger syndrome. In disorders of the reproductive system: gynandrism and gynism, aspermatogenic gynecomastia without aleydigism; male hypogonadism (sometimes with bulimia), postpuberal castration, menopause, ovarian disorder, paradoxical (Gilbert Dreyfus) disorder.
Otherwise induced:
By immobilization in adults and children; by psychic disturbance; by social and cultural pressure.

atic dysfunction, namely, increased secretion of insulin and probably increased secretion of glucagon. Such an etiology is supported by the fact that obese hyperglycemic mice show a sixfold increase in the rate of hepatic glycogen turnover and in hepatic phosphorylase activity. (Glucagon is known to activate hepatic phosphorylase activity.) Of particularly critical importance in the definition of metabolic obesity are the facts that in this syndrome, fasted rates of lipogenesis are increased over the fasted normal rates; fasting does not cause hyperketonemia; and reducing the animals to normal weight by underfeeding them does not bring body composition back to normal, but leaves them with a fat content still considerably greater than the normal fat content at the expense of non-fat tissues.

It is interesting to note that another type of genetic obesity in mice, also associated with hyperglycemia, has been described recently

TABLE II

Comparison Between Regulatory and Metabolic Obesities in Mice from the Same Litter

Object of Comparison	Obese Hyperglycemic Syndrome (Metabolic)	"Goldthioglucose Obesity" (Regulatory)
Etiology	Mendelian recessive	1 mg./gm. goldthioglucose
Pathology and mechanism	Pancreatic dysfunction, hyperplasia of islets of Langerhans, increased insulin and glucagon secretion.	Hypothalamic lesions: destruction of cells regulating intake in ventromedial area
Energy balance	Positive during moderate hyperphagia, moderate or small increase in O_2 consumption, activity is drastically decreased	Positive during considerable hyperphagia
Effect of type of diet	Maximum weight gain on high carbohydrate, less on protein, less or decreased on high fat.	Maximum weight gain on high fat diet, less on carbohydrate, decreased on high fat
Effect of weight reduction	Body composition remains obese: i.e. animal loses nitrogen as well as fat, but is still obese when weight is normal or below normal.	Brings body composition back to normal
Resistance to cold	Drastically reduced.	Normal
Blood glucose levels	Generally hyperglycemic; further increased by growth hormone, etc.	Normal
Total levels of blood lipids	Elevated	Elevated
Blood cholesterol levels	Elevated; further elevated by growth hormone, etc.	Normal
Effect of administration of hormones	Abnormal sensitivity to hyperglycemic effects of growth hormone, glucagon, etc. Increased resistance to insulin.	Normal
Mating behavior	Absent	Normal, though less frequent.
Lipogenesis *in vivo*	Increased with hyperphagia and increased during fasting. Fatty acids are broken down and resynthesized abnormally fast.	Increased with hyperphagia, normal during fasting. Normal fatty acid breakdown.
Hepatic lipogenesis *in vitro*	Increased with hyperphagia and increased during fasting.	Increased with hyperphagia, normal during fasting.
Adipose tissue metabolism	Glucose oxidation half of normal, impaired pyruvate metabolism	Glucose oxidation normal, pyruvate metabolism normal
Cholesterogenesis *in vivo*	Increased during fasting	Normal during fasting
Acetate pool and turnover	Increased pool; the rate of turnover was considerably increased	Normal
Liver glycogen turnover	Considerably increased	
Enzymatic activities	Increased liver phosphorylase	Normal phosphorylase
Intestinal absorption	Increased in proportion to increase in hyperphagia	Increased in proportion to increase in hyperphagia
Body composition and the size of specific organs	High body fat, decreased protein, cholesterol content increased with weight, enlarged liver, heart, pancreas, thymus, adrenals; decreased uteri, ovaries, brain.	High body fat, slightly increased protein; cholesterol content normal; enlarged liver, kidneys, ovaries and uteri
Retention of steroid hormones	Increased in proportion to increase in body fat	Increased in proportion to increase in body fat
Ketone levels of fed animals	Slightly increased	Slightly increased
Effect of fasting on levels of blood ketones	Decreased	Normal elevation of levels of blood ketones

by workers in New Zealand.[5] These "NZO mice" exhibit a syndrome which, in spite of certain resemblances, is different from the hereditary obese hyperglycemic syndrome. First of all, NZO mice mate and produce offspring in contrast to the mice we used which were obtained by mating non-obese carriers of the obese gene or by artificial insemination or ovum transplantation. Extensive metabolic studies have not yet been published by the discoverers of this syndrome but reaction of the levels of blood glucose to fasting appears quite

different in these animals. The usually high blood sugar levels in NZO mice may go even higher during fasting, instead of going down rapidly when food is withdrawn, as they do in mice with the hereditary obese hyperglycemic syndrome. In the NZO mice, low glucose values are observed during pregnancy, and very low values at parturition. Like the hereditarily obese hyperglycemic mice, the NZO mice show insulin resistance.

Mice made obese by grafting ACTH-secreting tumors evince a number of metabolic abnormalities. Levels of blood glucose are high in some of the animals. The blood sugars of all such mice show a remarkable stability under fasting conditions. Levels of liver glycogen also remain higher than normal under fasting conditions, doubtless reflecting more active gluconeogenesis because of increase in circulating corticosteroids. Hepatic glucose-6-phosphatase activity is increased while phosphorylase activity is normal, unlike the finding in the obese hyperglycemic syndrome.[6] As in other forms of metabolic obesity (and characteristic of the class), the rate of lipogenesis in these animals during fasting is greater than that in normal animals during fasting.[7,8] When these animals are reduced to normal weight, their fat content is still much higher than normal,[9] which is a typical characteristic of metabolic obesities. Another difference between regulatory and metabolic obesities is that the animals with the metabolic obesities tested thus far fail to show the normal rise in blood ketones which accompanies starvation.

Behavioral studies also emphasize the difference between regulatory and metabolic obesities in mice. Regulatory and metabolic types react differently to different diets. Similarly, the association with pathologic conditions differs between the two classes. This has been analyzed, in terms of the complex interrelationships between various types of obesity and diabetes, in a recent review on hyperglycemia.[10]

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The Obese Hyperglycemic Syndrome in Mice

Metabolism of Isolated Adipose Tissue *In Vitro*

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THE GENETIC, morphologic and metabolic features of the obese hyperglycemic syndrome of mice have been described by Dr. Jean Mayer.[1] Our studies are concerned with the metabolism of adipose tissues of these obese animals and of their non-obese litter mates, and represent a collaborative enterprise between Dr. Mayer's laboratory and the Baker Clinic Research Laboratory. This is a summary of the results of these studies. The methodology used has been described in other papers.[2–5] The adipose tissue preparation chosen was primarily the epididymal fat body of male mice, although mesenteric adipose tissue has also been used in some instances.

RESULTS

Figure 1 demonstrates the appearance of the epididymal adipose tissue preparations used, and compares them with the already extensively studied epididymal adipose tissue preparation from rats. In order to facilitate quantitative comparison, a metric scale and the testes have been included in each instance. In A, the epididymal fat body of the rat is shown on the left, still connected to the spermatic blood vessels, epididymis and testis. For incubation, the portion used is that distal to the spermatic blood vessels, as shown on the right. In B, the epididymal fat body of a non-obese Swiss mouse (left) is compared to that of a Swiss mouse which became obese following the injection of gold-thioglucose. It is interesting to note that the epididymal fat body of the normal mouse is almost as large as that of the rat (A), and that obesity induced by the administration of gold-thioglucose resulted in a marked increase in the size of the epididymal fat body without, however, causing other changes in its appearance.

The epididymal fat bodies illustrated in Figure 1C were obtained from a mouse with the obese hyperglycemic syndrome (right) and from a non-obese sibling (left). In contrast to the tissue from the gold-thioglucose–obese mouse, the tissue from the obese hyperglycemic animal appears to be not only larger and loaded with fat, but also denser and more pigmented than the tissue of the non-obese sibling. In response to palpation, the tissue is distinctly firmer.

The possible existence of qualitative rather than merely quantitative differences between adipose tissue obtained from obese and that obtained from non-obese mice of the obese hyperglycemic strain is further supported by the analytic data shown in Table I. As the degree of obesity increased the increased amount of epididymal adipose tissue was accounted for only in part by an increase in its fatty acid content. It is noteworthy that the nitrogen content

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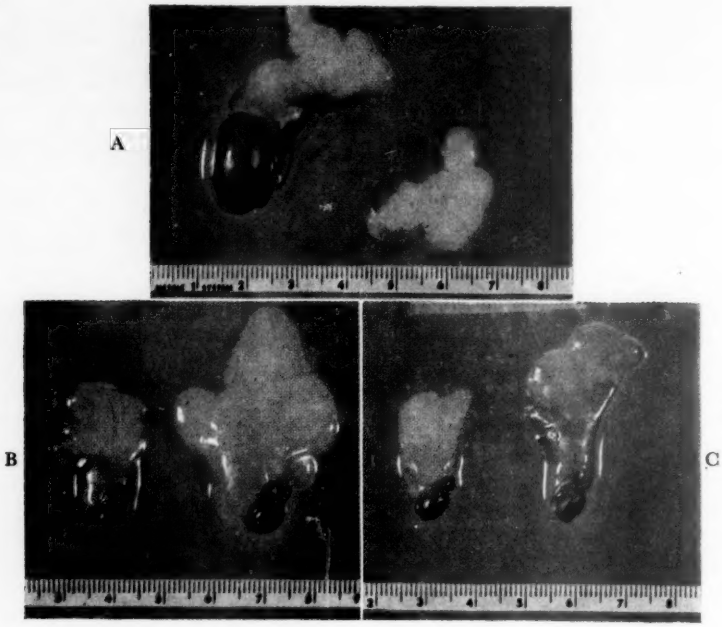

FIG. 1. Epididymal adipose tissue of **A**, a normal albino rat—the adipose tissue is shown connected with spermatic vessels and testis on the left and after being cut at the level of the spermatic vessels, on the right; **B**, a non-obese Swiss mouse (left) and a gold-thioglucose–obese Swiss mouse (right); **C**, a mouse with the obese hyperglycemic syndrome (right) and a non-obese sibling (left).

of the entire adipose tissue increased as the weight of the tissue increased; the per cent of nitrogen content decreased only slightly at first, and increased in the older animals. While some of the increased nitrogen in the adipose tissue may reflect the necessary growth of supporting structures (such as blood vessels), the data suggest that obesity in mice with the obese hyperglycemic syndrome may be accompanied by true hypertrophy of adipose tissue, be it an increase in the number of adipose tissue cells, or an increase in cytoplasmic adipose tissue mass, as has been previously suggested by Dr. Hausberger.[6] As also shown in Table I, the nitrogen increase in adipose tissue was less marked in gold-thioglucose–obese mice than it was in the mice with the obese hyperglycemic syndrome. This discrepancy between obese adipose tissue from obese-hyperglycemic and gold-thioglucose–obese mice was not present in mesenteric adipose tissue, although nitrogen determinations in mesenteric adipose tissue may be less meaningful because of the greater contamination of this tissue by lymphatic tissue and other non-adipose components.

Metabolism of glucose

Studies concerning the metabolism of glucose by adipose tissue from obese hyperglycemic mice and from the non-obese control animals are summarized in Tables II and Figures 2 and 3. When expressed in terms of unit of nitrogen content of adipose tissue obese adipose tissue uniformly utilized less glucose than did adipose tissue obtained from non-obese siblings. This was true both in the absence of added insulin and in the presence of increasing concentrations of the hormone. The sensitivity to insulin, however, did not appear to be grossly disturbed. These findings appear consonant with the diabetic syndrome characteristic of

TABLE I

Fatty Acid and Nitrogen Content of Epididymal and Mesenteric Adipose Tissue from Obese Mice*

Group of mice	State of Animals	Body Weight (gm.)	No. of Animals	Epididymal Adipose Tissue (Pad Pair)				Mesenteric Adipose Tissue	
				Weight (mg.)	Fatty Acid % (Wet wt.)	Nitrogen (mg.)	Nitrogen % (wet. wt.)	Fatty Acid % (wet wt.)	Nitrogen % (wet wt.)
Obese hyperglycemic	Control	23–35	15	470	77	0.75	0.16	53	0.36
	Obese	30–39	5	1,830	86	2.23	0.12	—	—
	Obese	40–49	14	2,650	84	3.11	0.12	81	0.10
	Obese	50–65	11	2,540	74	5.38	0.21	—	—
Obese-gold–thioglucose	Control	29–52	16	720	81	1.20	0.16	78	0.35
	Obese	50–68	11	2,620	81	2.43	0.09	85	0.13

* From: CHRISTOPHE, J., JEANRENAUD, B., MAYER, J. and RENOLD, A. E. *J. Biol. Chem.*, in press.[5]

TABLE II

Glucose Uptake by Epididymal Adipose Tissue from Obese Hyperglycemic Mice*

State of Animals	Mean Body Weight (gm.)	No. of Experiments	Response to Insulin (μU/ml.)			
			0	10^2	10^3	10^4
Non-obese	27	6	17.2 ± 3.2	23.3 ± 4.2	28.9 ± 5.6	46.7 ± 16.6
Obese	43	6	6.7 ± 1.0	8.3 ± 3.2	10.0 ± 2.3	15.0 ± 5.2

* Expressed as μM glucose per mg. N_2 over a three hour period. (Glucose Concentration = 20 mM/L.). Data from CHRISTOPHE, J., JEANRENAUD, B., MAYER, J. and RENOLD A. E. *J. Biol. Chem.*, in press.[5]

this type of experimental obesity, although it should be noted that glucose uptake when calculated in terms of the total tissue (rather than in terms of the unit of nitrogen content) results in increased rather than a decreased total glucose utilization by the obese tissue.

As was to be expected from the glucose uptake figures (Table II), studies with specifically labeled glucose similarity revealed decreased rates of metabolism of glucose carbons-1 and carbon-6 by epididymal adipose tissue from obese hyperglycemic animals (Fig. 2). When expressed in terms of unit of nitrogen content of the tissue, the oxidation of glucose carbon-1 and glucose carbon-6, as well as the incorporation of these glucose carbons into fatty acids, glycerol and glycogen, was approximately one-half that of the control tissues. Other than this over-all decrease in glucose metabolism, however, the results obtained failed to suggest the presence of a characteristic distortion of the pattern of glucose carbon metabolism by obese adipose tissue when compared with tissue from non-obese animals. In particular, glucose carbon-1 was oxidized in excess of glucose carbon-6 in all tissues, and lipogenesis from carbon-6 consistently exceeded lipogenesis from carbon-1 in tissues of both obese and non-obese animals.

Again in accordance with the data on glucose uptake (Table II), the presence of insulin clearly augmented the metabolism of glucose carbon-1 and glucose carbon-6 by both obese and non-obese adipose tissue *in vitro*. The absolute values reached for the obese tissues were, consistently, approximately one-third as great as those reached for non-obese tissues, with the possible exception of the oxidation of

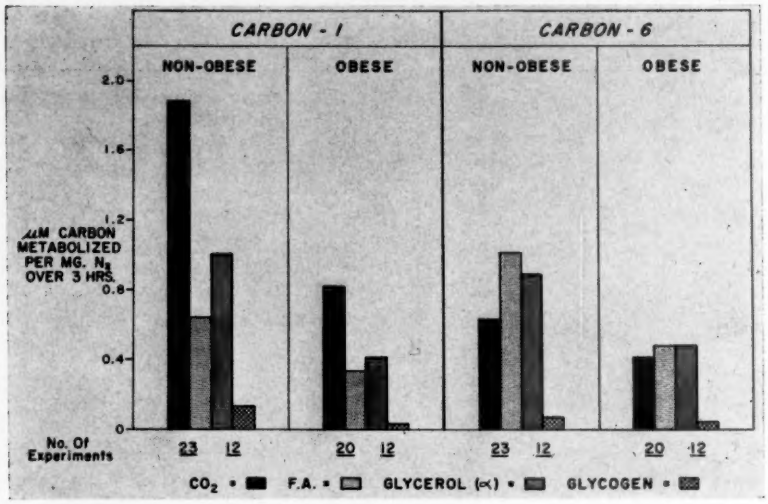

FIG. 2. Metabolism of glucose-1-C^{14} and glucose-6-C^{14} by epididymal adipose tissue from obese hyperglycemic mice and their non-obese siblings. F. A. = Fatty acid in this and the following illustrations.

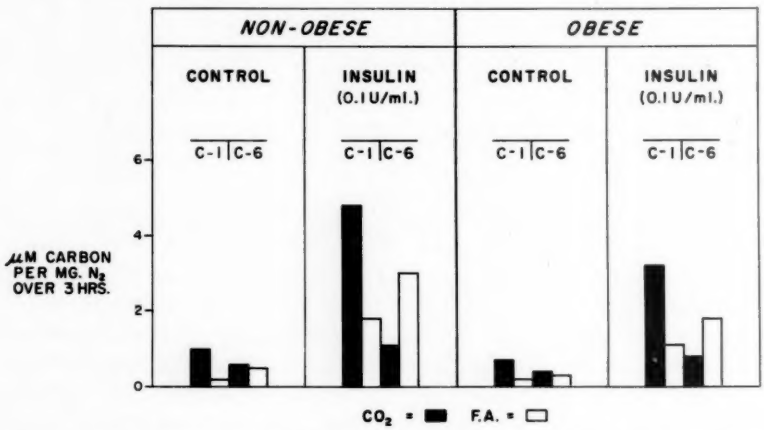

FIG. 3. Effect of insulin on the metabolism of glucose by epididymal adipose tissue of obese hyperglycemic mice and their non-obese siblings.

glucose carbon-6, although we have not as yet established whether or not this exception is truly significant and reproducible. The uniformly depressed glucose metabolism by obese adipose tissue, which is characteristic of the obese hyperglycemic syndrome just described, does not appear to be equally characteristic of obesity associated with hyperphagia resulting from hypothalamic lesions (i.e., of obesity induced by the administration of gold-thioglucose). When tissue, obtained from the animals in which obesity was induced, was compared with tissue obtained from non-obese animals of the same strain, only a mild decrease (if any) in the glucose uptake and a decrease by less than one-third in the metabolism of glucose carbon-1 and carbon-6 was observed (Fig. 4), in contrast to the depression by at least one-half and frequently two-thirds which was observed in adipose tissue from obese

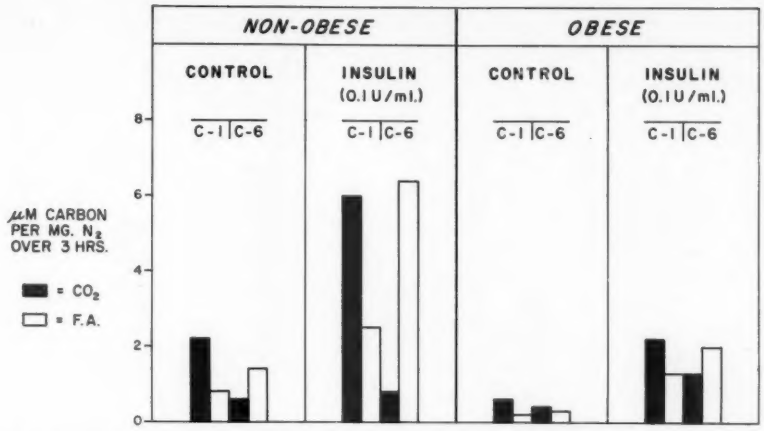

FIG. 4. Effect of insulin on the metabolism of glucose by epididymal adipose tissue of gold-thioglucose–obese mice and non-obese control mice.

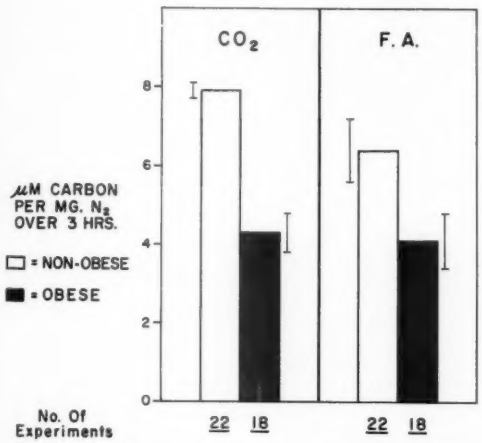

FIG. 5. Metabolism of pyruvate-2-C^{14} by epididymal adipose tissue of obese hyperglycemic mice and their non-obese siblings. (Pyruvate concentration 40 mM/L.)

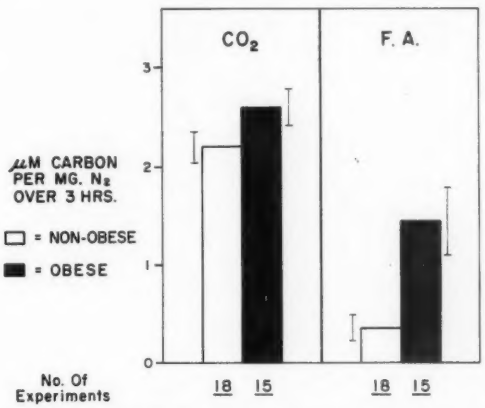

FIG. 6. Metabolism of acetate-1-C^{14} by epididymal adipose tissue of obese hyperglycemic mice and their non-obese siblings. (Acetate concentration 60 mM/L.)

hyperglycemic animals (Fig. 3). Gold-thioglucose–obese animals do not present a diabetic syndrome.

Metabolism of Pyruvate

When both oxidation and lipogenesis were expressed in terms of units of nitrogen content in the tissue, adipose tissue from obese hyperglycemic mice oxidized less pyruvate-2-C^{14} and incorporated less of its carbon into fatty acids, than did adipose tissue from non-obese siblings (Fig. 5). This decreased lipogenesis from pyruvate, however, was much less marked than that reported for lipogenesis from glucose (Figs. 2 and 3).

Metabolism of Acetate

A striking discrepancy appears between the metabolism of glucose and of pyruvate and that of acetate by adipose tissue from obese hyperglycemic mice. Adipose tissue from the obese siblings utilized less glucose and pyruvate carbon, both for oxidation and for lipogenesis, whereas, acetate oxidation was found unimpaired and lipogenesis from acetate was significantly enhanced (Fig. 6). In order to rule

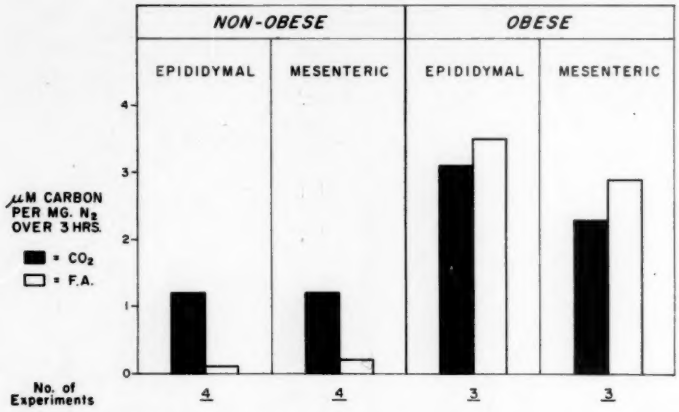

FIG. 7. Metabolism of acetate-1-C^{14} by epididymal and mesenteric adipose tissue from obese hyperglycemic mice and their non-obese siblings.

out simple geometric factors (e.g., altered diffusion into the obese tissue) the same comparison was carried out in another group of animals, using both epididymal and mesenteric adipose tissue. The mesenteric adipose tissue of obese hyperglycemic animals approximates, in terms of appearance and tissue thickness, the epididymal adipose tissue of non-obese animals. As shown in Figure 7, both epididymal and mesenteric adipose tissue from obese hyperglycemic animals, in this series of experiments, exhibited doubling of acetate carbon oxidation and increased lipogenesis from acetate by a factor between 10 and 15. Furthermore, when lipogenesis from acetate was similarly compared in both mesenteric and epididymal adipose tissue obtained from gold-thioglucose–obese mice and from the non-obese control animals, no striking acceleration of lipogenesis from acetate *in vitro* was observed.

COMMENTS

In a comparison of adipose tissue from obese hyperglycemic mice with tissue obtained from their non-obese siblings, three major and possibly important discrepancies have been noted. First, analysis of the tissues for fatty acids and nitrogen revealed increasing amounts of nitrogen in the tissue with increasing accumulation of lipids. Since no significant decrease in nitrogen concentration was observed, it would seem likely that in mice with the obese hyperglycemic syndrome the cytoplasmic mass of adipose tissue increases. Secondly, over-all glucose utilization, as well as glucose metabolism into all metabolic products measured, was less than that of tissue obtained from the non-obese siblings, when compared on the basis of either unit of tissue weight or unit of nitrogen content in the tissue. This observation seems to be consistent with the hyperglycemia of these animals. Lipogenesis from pyruvate was also decreased, although it was less pronounced.

The third and most striking deviation from normal in the epididymal adipose tissue obtained from obese hyperglycemic animals concerns the incorporation of acetate carbon into fatty acids. When compared on the basis of unit of nitrogen content in the tissue, lipogenesis from acetate was increased between five and ten-fold even though oxidation of acetate to CO_2 by the same tissue either remained unchanged (Fig. 6) or increased to a much lesser degree (Fig. 7). Although total acetate utilization by obese tissue could not be measured, the differential behavior of acetate oxidation and lipogenesis from acetate suggests that the greatly accelerated rate of incorporation of acetate into fatty acids is not the result of differential dilution in acetate or acetyl coenzyme A pools of different size.

Summarized in Figure 8 are the results concerning the activity of obese and normal adipose tissue with regard to the incorporation of sub-

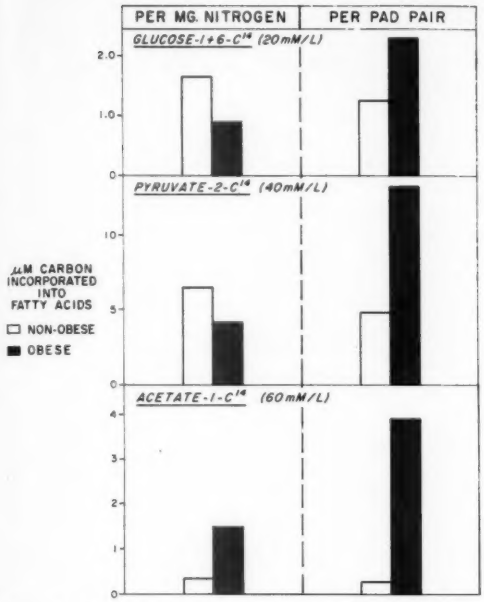

FIG. 8. Lipogenesis *in vitro* by epididymal adipose tissue (per pad pair) from obese hyperglycemic mice and their non-obese litter mates. (From: CHRISTOPHE, J., JEANRENAUD, B., MAYER, J. and RENOLD, A. E. *J. Biol. Chem.*, in press.[5])

strate carbon into fatty acids. On the left of the illustration the comparison has been carried out in terms of unit of nitrogen in the tissue, while on the right, the comparison is in terms of total epididymal adipose tissue for each animal. It now becomes evident that total lipogenesis by this tissue is increased from all substrates although most markedly from acetate. We are thus confronted with the paradoxical situation of increased utilization of substrate carbon for lipogenesis in the face of good evidence for decreased glucose utilization. Simple diffusion factors seem to be ruled out by the results of the experiments with epididymal and mesenteric adipose tissue (Fig. 7) and also by the failure to observe this tendency to accelerated lipogenesis from all substrates in equally obese tissue from gold-thioglucose–obese mice. Although these results should be interpreted with great caution at this time, they do suggest that adipose tissue from obese hyperglycemic mice metabolizes an excessive proportion of available substrate carbon into fatty acids and, furthermore, that this acceleration does not appear to be dependent upon the simultaneous occurrence of accelerated glucose metabolism. Observations consistent with this interpretation have been reported in intact animals[7,8] and isolated liver.[9]

Studies with glucose specifically labeled in positions 1 or 6 failed to reveal the existence of a characteristic anomaly in the pattern of glucose metabolism, subsequent to its entrance into the glucose-6–phosphate pool.

SUMMARY

The metabolic activity of epididymal adipose tissue from obese hyperglycemic mice was compared with that of adipose tissue from their non-obese siblings. Three main differences were noted. (1) Adipose tissue from obese animals contained more nitrogen, suggesting an increased cytoplasmic mass. (2) Glucose utilization and metabolism by adipose tissue from obese hyperglycemic mice were persistently less than that of control tissues, when the comparison was carried out on the basis of nitrogen content or wet weight. This was especially true for tissue from the most obese mice. (3) Incorporation of acetate carbon into fatty acids was five to ten times greater than normal in tissue from obese animals, whereas acetate oxidation to CO_2 was essentially the same as in tissue from non-obese control animals.

Tissues from obese and non-obese animals did not differ qualitatively in their response to insulin or growth hormone, and studies with specifically labeled glucose did not suggest the presence of abnormal pathways of glucose metabolism in the obese tissue.

These findings suggest that adipose tissue from obese hyperglycemic mice metabolizes an excessive amount of available substrate carbon to fatty acids, and that fatty acid synthesis in this tissue is less dependent upon the simultaneous occurrence of accelerated glucose metabolism than is true for normal adipose tissue from mice or rats.

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DISCUSSION

Dr. John A. Moorhouse (*Winnipeg, Canada*): We have recently been interested in performing studies on pyruvate metabolism in obese hyperglycemic people. We have done this by development of a pyruvate tolerance test.

Briefly, this is accomplished by injecting 10 gm. of sodium pyruvate into the patient and plotting the levels of blood pyruvate, at two and a half minute intervals for fifteen minutes (beginning fifteen minutes after the injection). We find that if we plot, as one does for a glucose tolerance test, the logarithm of the increment of pyruvate over the initial fasting pyruvate level, this produces a straight line, and we can express the slope of this line as a regression coefficient.

The slope of the line, or the regression coefficient, for non-diabetic people is approximately $-.041$; it is not influenced by age or sex. It is not influenced by obesity if the obese person does not have diabetes.

When we study obese people who do have hyperglycemia, we find that the slope of this line is elevated and that the regression coefficient is approximately .030.

As to this impaired rate of fall of the levels of pyruvate after injection, the difference between the two is highly significant.

It is interesting that the rate of pyruvate turnover is not improved after we correct the hyperglycemia in these individuals by administration of insulin for a number of days. The mean for the group is exactly the same as it was before treatment. This may indicate a metabolic defect in these persons which is not corrected by insulin. Lack of insulin is not responsible; therefore, it depends on some other mechanism.

Dr. William Parson (*Charlottesville, Virginia*): As I mentioned yesterday, Dr. Hollifield, in our laboratory, has found results identical to those reported by Dr. Renold, using tagged acetate and studying the formation of fats *in vitro*, using a congenitally obese strain of mice. It is of interest that we used females and studied the inguinal subcutaneous fat and obtained the same results. Our controls, which were also the obese-gold–thioglucose mice, did not show this. The mice treated with compound A pellets also had this increased synthesis from acetate.

Dr. Jay Tepperman (*Syracuse, New York*): I would like to interpose a question, if I may. Has adipose tissue been studied in the animals which are destined to become fat but which have not become fat as yet?

Dr. Renold: Yes. I did not go into that here because it is difficult to chart. However, in the report which Dr. Christophe has prepared, all of the data concerning the obese hyperglycemic mice have been calculated for three groups, according to body weight. The mice of the first group were just beginning to gain weight and, indeed, there was hardly any mean weight difference between this group and the control group. However, the interesting thing is that some of the differences between obese and non-obese tissue were most marked in this group whereas the abnormalities of glucose metabolism were relatively mild at this stage. The increased rate of acetate incorporation into fatty acids was greater in these just obese mice than in the more markedly obese animals. The diabetic pattern of glucose metabolism distinctly gets more severe with increasing weight.

Dr. Tepperman: I think this is a particularly exciting discovery, because it has always been difficult in the past, when one found metabolic abnormalities in an animal after the obesity had become established, to know to what extent this was due to some sort of adaption. Here I think we have a clear cut example of something which was present in the beginning.

Dr. Theodore B. Van Itallie (*New York, New York*): I do not recall Dr. Mayer's mentioning it, but I think that in his studies he has shown that the obese hyperglycemic mouse is insulin-resistant. This seems to me to fit into Dr. Renold's observations that insulin-like activity is increased and the blood sugar, nevertheless, remains elevated.

I wonder whether he might interpret this as being a resistance of the tissue.

Dr. Renold: I do not know how one can really settle that problem of insulin-resistance. The only way that I can prove a tissue or even a person to be insulin-resistant is to demonstrate that the insulin effect appears at a higher dosage than is true in the non-resistant. This does not seem to be true in adipose tissue of the obese mice, although the magnitude of the effect at a given dose is less.

Now, the reason for that may be entirely remote from the action of insulin. If you strike a chord on a piano, the sound is different from that of the same note on a xylophone. It may not have anything to do with the effect of insulin, *per se*.

DR. MAYER: I think there may be some indirect indications, though, that there is a definite difference in the tissues, at least in two respects.

The first one is that you can eventually bring the blood sugar of the obese mice down to extremely low levels if you give them much larger doses of insulin than you would to the non-obese mice. What happens to the animals then is completely different from what happens to non-obese animals. For example, we have never seen convulsions in these animals. The reaction to hypoglycemia appears to be very different.

The other difference is that these animals will recover from hypoglycemia caused by insulin even though the blood glucose may have been brought down to levels from which non-obese animals never recover.

Although these are indirect indications, I think that they tend to suggest that there must be some tissue factors which are quite different in the reaction to insulin.

DR. R. H. WILLIAMS (*Seattle, Washington*): One extra factor that could account for the lesser effectiveness of a given amount of insulin is the fact that it has been demonstrated at Brown University (working with this same strain, I believe), that there is an increase in the insulinase activity. Therefore, more insulin would be degraded.

DR. ALBERT I. WINEGRAD (*Philadelphia, Pennsylvania*): I would like to ask Dr. Renold a few questions concerning this remarkable finding with regard to acetate.

In view of the fact that you have demonstrated a decreased glucose uptake, it is rather remarkable to find an increased fatty acid synthesis from acetate and from pyruvate, under these circumstances. Essentially, what we are doing here is comparing incorporation in different animals. I would be a little bit more at ease if you could answer these questions.

First of all, is there an effect of insulin in this tissue on fatty acid synthesis from acetate or pyruvate, in the absence of glucose?

Secondly, does glucose stimulate fatty acid synthesis from acetate or pyruvate? And, as we showed in our earlier experiments, does pyruvate stimulate the incorporation of acetate into fatty acid?

One would be willing to accept a peculiar and, in this case, unique type of fatty acid synthesis in the adipose tissue of this animal if one could show that this differed in any way from the normal. Otherwise I think we would have to be worried about the fact that this really might be due to a difference in permeability.

DR. RENOLD: I can assure you that you would not be the only one who would be more at ease if we knew of a mechanism explaining this increased acetate incorporation. There is a definite discrepancy between decreased glucose utilization and, in the same tissue, increased synthesis of fatty acids from acetate, not from glucose.

There is no insulin effect on acetate itself. There is no insulin effect on pyruvate. There is a perfectly good insulin effect, in the presence of glucose, on either of those two, even though we must assume that the glucose uptake is less than in the control tissue.

The glucose effect, *per se*, without insulin is quite good, too, even though, again, one must assume that less glucose is going in than in the control tissue. However, in those particular experiments we did not measure it.

You can look at this in two ways. One is that we should look for a possibly unusual mechanism of fat synthesis in this tissue, but, thus far, we have been unable to find one. Pyruvate-1-C^{14}, -2-C^{14}, and -3-C^{14} do just what they ought to do. Acetate-1 and 2 do what they ought to do. We have not studied the possibility of abnormalities of the propionate or butyrate precursors thoroughly, but so far we have not found any. Therefore, the discrepancy between decreased glucose utilization and increased synthesis from acetate is unexplained.

Also, there is no evidence in the pattern which would suggest that there is a greater proportion of glucose utilization by a pathway favorable for fatty acid synthesis, which would be an alternative explanation.

DR. WINEGRAD: What would happen in these animals if alloxan-diabetes were induced in them? Does fatty acid synthesis from pyruvate and acetate continue to occur in the presence of impaired glucose metabolism? This would be a unique situation.

DR. MAYER: The difficulty in working with these animals is that they die almost immediately in a state of hypoglycemia when they are pancreatectomized.

Two things which may be germane to your question are these. If you put these animals on a high fat and carbohydrate-free diet, their fat synthesis from acetate is extraordinarily depressed, *in vivo*. It is depressed to levels which are as low as those which occur when the animals are fasted.

Fat-Mobilizing Activity of Human Urine Extract

T. M. CHALMERS, M.D., F.R.C.P., G. L. S. PAWAN, B.SC., AND A. KEKWICK, M.A., M.B.

IN 1947 Weil and Stetten[1] reported that an alkaline extract of urine from fasting rabbits was effective in increasing liver fat in mice. More recently[2,3] we have shown that fat-mobilizing activity is present in human urine under certain conditions including fasting.

CONDITIONS IN WHICH ACTIVITY IS FOUND

The conditions in which we have found activity in the urine are shown in Table I. Carbohydrate deprivation appears to be at least as important a stimulus as calorie deficiency. With diets of 1,000 calories, activity appears when the carbohydrate content is reduced below 100 gm. and increases progressively with further carbohydrate restriction.

In two patients with diffuse lipoatrophy, activity has been found in the urine although a normal mixed diet was being consumed. One of them was an adolescent girl with well controlled diabetes and accelerated growth in addition to lipoatrophy. The other patient was not diabetic.

As Table I also shows, activity has been found in diabetic ketosis, in widespread carcinoma with wasting, and during the first few days after major surgical operations. In all these patients the intake of calories was relatively low and there is some doubt about the interpretation of the finding.

A normally functioning anterior pituitary appears to be necessary for the production of the active material. Six patients with deficient function of the anterior pituitary, fasting for thirty-six hours or on a diet of 1,000 calories and 90 per cent fat, failed to produce any activity in the urine (Table III).

BIOLOGICAL EFFECTS

The effects of subcutaneous injection of active material into mice are summarized in Table II. Liver fat, blood lipids and blood ketones all increase, the effects being maximal about six hours after injection (Fig. 1, Table III). Values in this and subsequent tables are means ± standard error of the mean. The minimal dose of our most active extracts for obtaining the liver fat and ketone effects is about 10 μg. for each mouse. Larger doses ($> 200\mu$g.) are needed to produce unequivocal rises in blood lipids (total lipids, phospholipid, cholesterol and non-esterified fatty acids).

There is an early and transient fall in blood sugar (Fig. 1). Utilization as well as mobilization of body fat is increased. This has been demonstrated using labeled tripalmitate.[2] However, it is demonstrated more simply by the loss of body weight and carcass fat after single large doses or repeated small ones (Figs. 2 to 4, Table IV). No change in appetite and food intake accompanies this increased catabolism of body fat. On the simplest assumption, therefore, total expenditure of energy must increase. Alternatively, the efficiency of the utilization of energy may diminish. Measurements of the uptake of oxygen are in progress.

PREPARATION OF EXTRACTS

Our present method of extraction is illustrated in Figure 5. The preparation of the alkaline extract may be conveniently carried

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Presented at the Brook Lodge Invitational Symposium on Energy Balance, sponsored by The Upjohn Company, September 29, 1959, at Brook Lodge, Augusta, Michigan.

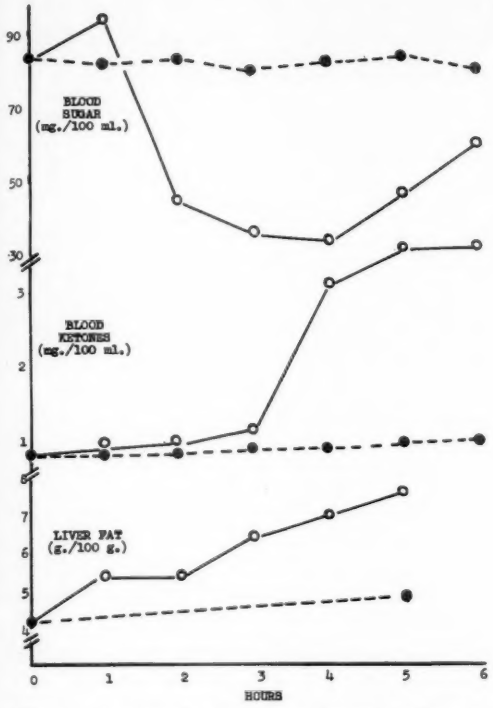

FIG. 1. Time-course of effects of fat-mobilizing substance (FMS) on blood sugar, ketone bodies (as acetone), and liver fat in mice. Groups of animals killed at hourly intervals after subcutaneous injection. Broken lines indicate results in control animals injected with saline.

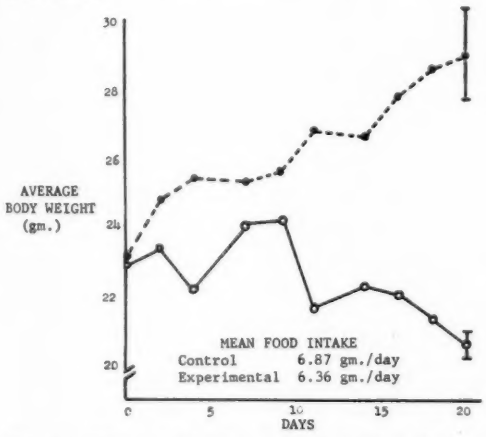

FIG. 2. Effect of injection of saline (bróken line) or active extract (solid line) three times weekly for twenty days on body weight of mice (ten mice in each group). The difference in food intake is the largest observed in six experiments. For carcass analysis see Table IV.

TABLE I
Fat-Mobilizing and Ketogenic Activity in Urine

Present (Fasting)	Absent (Normal Diet)
1,000 Calories, 90 per cent Fat	1,000 Calories, 90 per cent CHO
1,000 Calories, 90 per cent Protein	Lipodystrophy } non-fasting
2,000 Calories, 83 per cent Fat	Late Pregnancy } non-fasting
Diffuse lipoatrophy (non-fasting)	Brief Exercise } non-fasting
Diabetic ketosis*	
Carcinomatosis*	
Postoperative*	

* Calorie intake low.

TABLE II
Effects of Active Extracts in Mice

Increased	Diminished
Liver fat	Blood sugar
Blood lipids	Body weight
Blood ketones	Carcass fat
Fat utilization	

TABLE III
Effects of Urine Extracts from Normal and Pituitary-Deficient Subjects on Liver Fat and Blood Ketones in Mice

Material Injected Six Hours Previously	Liver Fat (gm./100 gm.)	Blood Ketones (mg./100 ml. as acetone)
Saline	4.3 ± 0.14	1.68 ± 0.37
NFU	4.8 ± 0.24	2.10 ± 0.46
FU	7.2 ± 0.33	4.08 ± 0.45
FU (Hypopituitarism)	3.9 ± 0.17	1.35 ± 0.33

NOTE: NFU = Non-fasting urine extract. FU = extract of urine collected during fasting or 90 per cent fat, 1,000 calorie diet. Hypopituitarism = eight observations on six subjects, one hypophysectomized three weeks previously, two with postpartum pituitary necrosis and three with chromophobe adenomas. All patients were receiving maintenance doses of cortisone and thyroid.

out in an M.S.E. basket centrifuge. The use of oxycellulose (Eastman) increases the potency twenty to thirty times (compared with the ultrafiltrate). Our extract is shaken with 25 to 50 per cent of oxycellulose overnight

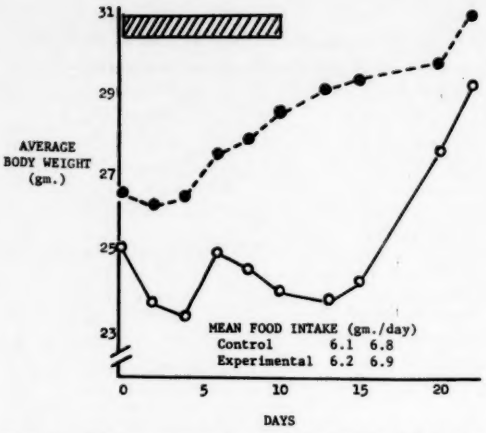

FIG. 3. The same experiment as in Figure 2 but the injections were stopped after ten days. Note recovery of body weight in treated group. No difference in food intake between control and experimental groups in either part of the study.

(sixteen hours). About 10 per cent of the original activity is not extracted during this single exposure: two or more extractions therefore will be needed for quantitative work. The yield for the excretion of urine over a twenty-four hour period has ranged from 1 to 7 mg.

TABLE IV

Carcass Analysis After Treatment with Active Extract for Three Weeks (gm./100 gm.)

Substances Analyzed	Control	Experimental	Average Net Change (gm./mouse)	Average Net Change (gm./100 gm.)
Fat	14.3 ± 0.5	9.9 ± 0.4	−1.25	−38
Water	64.1 ± 0.7	67.3 ± 0.4	−0.80	−5.5
Protein	11.8 ± 0.4	13.5 ± 0.3	+0.07	+2.5

NOTE: The same experiment as illustrated in Figure 2. The amount of water lost is somewhat greater than can be accounted for by depletion of fat depots. We have not observed any significant gain or loss of protein after prolonged treatment with active extracts.

CHEMICAL PROPERTIES

The oxycel extract has a nitrogen content of about 8 per cent. After acid hydrolysis, it yields the following amino acids: histidine, phenylalanine, leucine, serine, cystine, aspartic acid and a trace of alanine. It gives only a weakly positive Molisch test for carbohydrate material.

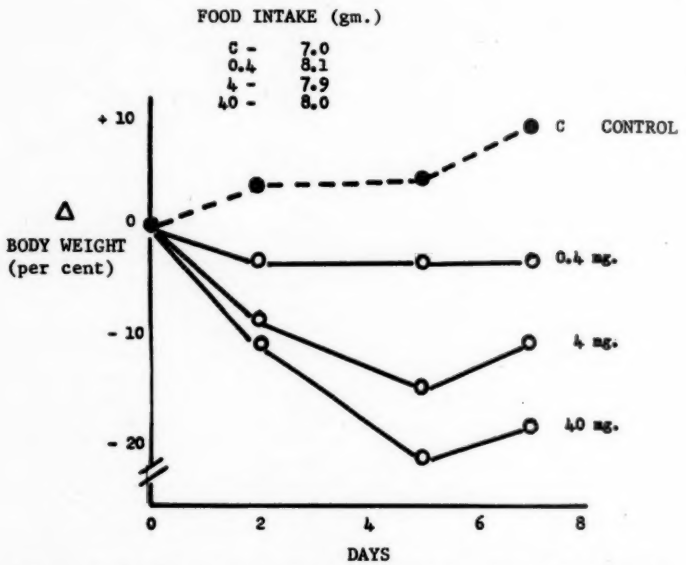

FIG. 4. Body weight as per cent of initial value plotted against time in days. Single mice receiving graded single doses of oxycel material. Fat content of carcasses on day 7: Control, 14.9 per cent; 0.4 mg., 12.4 per cent; 4 mg., 11.7 per cent; 40 mg., 10.3 per cent.

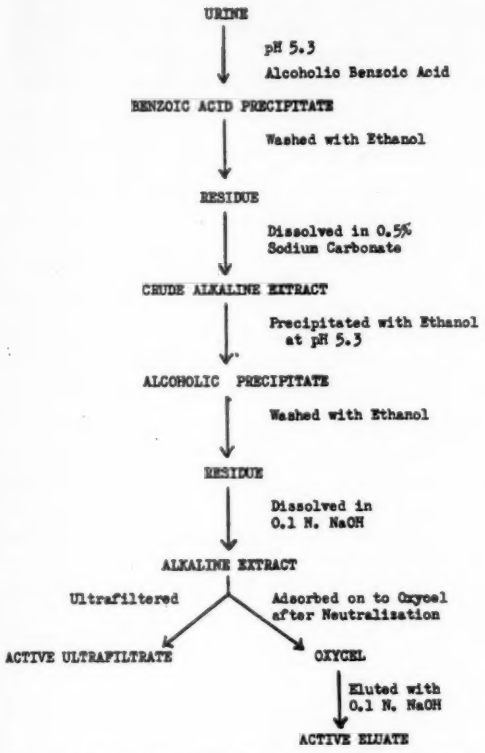

FIG. 5. Extraction procedure. The final eluate is neutralized and freeze-dried.

The biologically active material is thermostable up to 80°c. in 0.1 N alkali. It is destroyed by boiling for two minutes. It is ultrafiltrable through Visking membrane (i.e. molecular weight less than 18,000). After peptic digestion some activity remains, but activity is completely destroyed by trypsin and by chymotrypsin.

EFFECT ON ADIPOSE TISSUE *IN VITRO*

We have used a modification of the method of White and Engel.[4] Pieces of epididymal fat weighing about 50 mg. from rats weighing 90 to 110 gm. have been incubated in a bicarbonate medium containing 4 per cent bovine albumin. Non-esterified fatty acid (NEFA) release into the medium during a three hour incubation has been determined by Dole's[5] method. Addition of oxycel material increases NEFA release (Table v), the threshold concentration being 1 μg./ml. The response is linearly related to the logarithm concentration. Similar data have been obtained using the cruder ultrafiltrate preparation at concentrations about twenty times greater.

TABLE V

Release of Non-esterified Fatty Acid (NEFA) from Adipose Tissue Incubated with Oxycel Extract

Concentration of Extract (μg./ml.)	NEFA Released (μM./100 mg.)*
0 (18)†	0.18 ± 0.08
0.06 (4)	0.23 ± 0.16
0.6 (4)	0.44 ± 0.10
1.6 (5)	1.03 ± 0.39
6 (4)	2.20 ± 0.31
120 (3)	5.03 ± 0.45

* Values for NEFA are mean ± standard error of the mean.

† Figures in parentheses show the number of observations.

TABLE VI

Comparison of Effects of Urinary Material and Corticotropin on Blood Ketones, Liver Fat and Eosinophil Count in Mice*

Material Injected Six Hours Previously	Blood Ketones (mg./100 ml. as acetone)	Liver Fat (gm./100 gm.)	Eosinophils (cu./ mm.)
Saline	2.45 2.60	4.00 3.75	165 149
Oxycel Extract (50 μg.)	4.60 5.15	7.40 7.85	145 141
ACTH (0.5 U.)	3.75 4.15	5.95 6.50	75 60

NOTE: Absence of effect on eosinophils of urinary extract in a dose sufficient to produce relatively large effects on blood ketones and liver fat.

* Two mice per group.

RELATION TO CORTICOTROPIN AND GROWTH HORMONE

The active urinary substance has certain properties in common with corticotropin, namely, affinity for oxycellulose, lipolytic effect *in vitro* and capacity to lower blood

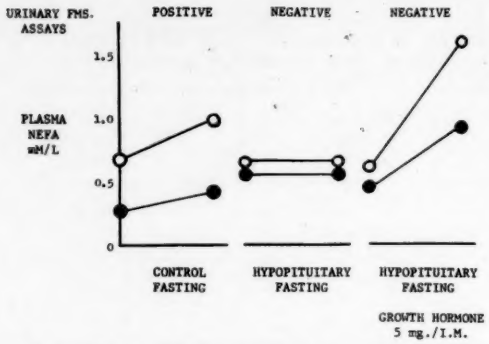

FIG. 6. Plasma NEFA concentrations at 8 A.M. and at noon in subjects fasted overnight: First column, two normal subjects; second column, two subjects with pituitary deficiency, one was recently hypophysectomized and the other had a chromophobe adenoma; third column, effect of human growth hormone (5 mg.) administered intramuscularly (I.M.) in the pituitary-deficient subjects. Urine collected between 8 A.M. and noon was assayed for fat-mobilizing substance (FMS). Activity was present in the normal fasting urines but absent in the urine from the pituitary-deficient subjects even after the growth hormone was administered. Urine collected for several days after administration of growth hormone was also negative.

sugar and to increase liver fat and blood ketones. The most obvious point of difference is in the effect on body weight and carcass composition. The urinary material also appears to be more stable in alkali. It does not depress the eosinophil count in the mouse (Table VI). See also Chalmers et al.[3]

This substance shares with the growth hormone the properties of lowering blood sugar and of increasing fat mobilization and catabolism. With respect to NEFA release it is more active *in vitro* and possibly less active *in vivo*. It does not increase the rate of growth nor the deposition of protein. It differs from the growth hormone also in its chemical properties, especially in its ultrafiltrability and its affinity for oxycellulose. We have examined the possibility that it is a fragment derived from the growth hormone. In two pituitary-deficient persons, urine collected after an intramuscular injection of the human growth hormone* has been assayed for fat-mobilizing activity. In neither case could any activity be detected by *in vitro* or *in vivo* methods (Fig. 6).

SUMMARY

In people who are actively mobilizing and utilizing fat, a substance can be extracted from the urine which will cause increased fat mobilization and catabolism in mice, with depletion of the body fat stores. The material is active *in vitro* in releasing NEFA from adipose tissue at a concentration of less than 1 μg./ml. The pituitary is necessary for its production. It is not corticotropin or the growth hormone. Its relation to these hormones is briefly discussed.

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* The human growth hormone was kindly supplied by Professor F. G. Young through the Medical Research Council.

Factors Concerned with the Regulation of Fatty Acid Metabolism by Adipose Tissue

GEORGE F. CAHILL, JR., M.D.,* BERNARD LEBOEUF, M.D.† AND ALBERT E. RENOLD, M.D.‡

As EVIDENCED by the number of articles appearing in the scientific literature, both clinical and basic, much attention has recently been directed toward the metabolism of adipose tissue. This tissue was long considered an inert site of energy storage except by investigators such as Wertheimer and Shapiro,[1,2] Stetten,[3] and Hausberger.[4,5] It has recently been shown that adipose tissue releases lipid as fatty acids§, and thereby provides a major, perhaps the major metabolic fuel, as recently reviewed by Frederickson and Gordon.[6] Adipose tissue has also been shown to be acutely and exquisitely sensitive to many hormones such as insulin[4–15] and epinephrine,[1,16–18] and, in addition, its sensitivity has been shown directly or indirectly to growth hormone,[19,22] adrenocorticotropic hormone,[22,23] glucagon,[23] thyroid hormone,[24] thyroid stimulating hormone,[22,25] and prolactin.[21]

The purpose of this paper is to summarize observations made at the Baker Clinic Research Laboratories on some of the metabolic events associated with the uptake and release of fatty acids in adipose tissue and the effect of various hormones on these mechanisms. The experiments were all performed under identical conditions, as described previously,[9–11] namely, incubation of epididymal fat pads (excised from rats) for three hours at 37°c. in Krebs-Ringer bicarbonate buffer containing 0.5 mM human albumin‖ and 5 mM glucose and equilibrated with 5 per cent CO_2:95 per cent O_2. The albumin was not extracted to remove fatty acids and contained approximately 1.2 M fatty acids per M albumin.

FATTY ACID EXCHANGE

Previous studies[26] have shown that adipose tissue obtained from normally fed rats and incubated in a medium containing about 0.6 mEq. per L. fatty acids is approximately in a steady state (Fig. 1) with the concentration of fatty acids in the medium. In other words, there is no change in the concentration of fatty acid in the medium during the period of incubation. Addition of a trace amount of highly active palmitate-1–C^{14} to the albumin in the medium prior to incubation resulted in a recovery of label in tissue triglyceride at the end of the incubation (Fig. 2). Therefore, there must have been a concomitant release of unlabeled fatty acids from the tissue into the medium equal to the quantity of labeled fatty acids taken up from the medium. Other studies demonstrated that if glucose was omitted from the medium, the incorporation of labeled palmitate-1–C^{14} into tissue triglyc-

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§ "Free" fatty acids or non-esterified (NEFA) or unesterified (UFA) fatty acids.

‖ Obtained through the courtesy of Dr. R. J. Pennell of the Protein Foundation, Inc.

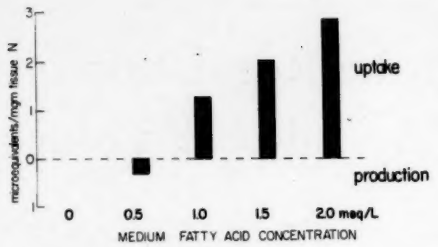

FIG. 1. Change in medium titratable fatty acids as a function of fatty acid concentration (glucose 5 mM). At approximately 0.6 mEq. per L. medium fatty acid concentration there is neither uptake into nor production of fatty acids by adipose tissue after three hours incubation under conditions described in the text. (From: Bally, P., Cahill, G. F., Jr., Leboeuf, B. and Renold, A. E. *J. Biol. Chem.*, 235: 333, 1960.[26])

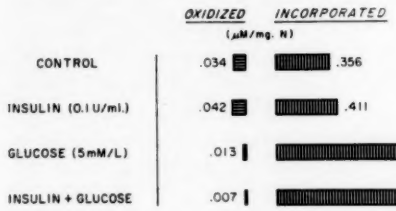

FIG. 2. Comparison of insulin and glucose on palmitate-1-C^{14} metabolism in adipose tissue. (From: Bally, P., Cahill, G. F., Jr., Leboeuf, B. and Renold, A. E. *J. Biol. Chem.*, 235: 333, 1960.[26])

eride was markedly reduced. In addition, the tissue was no longer in a steady state with the medium, but released more fatty acids than those incorporated, and caused an elevation in the concentration of fatty acid in the medium. These experiments suggest that glucose accelerated the process of esterification. This will be discussed later in this report.

EPINEPHRINE

With knowledge of the marked sensitivity of adipose tissue to epinephrine and norepinephrine, as reflected by fatty acid mobilization,[17] experiments were performed with glucose-C^{14} as substrate. It was expected that the mode of action of epinephrine might be elicited by a decreased rate of fatty acid esterification, thus disrupting the steady state in favor of the release of fatty acid. Surprisingly, the incorporation of glucose-C^{14} into glyceride-glycerol was increased five or six times by

TABLE I

Relative Metabolism of Glucose (5 mM) and Glycerol (5 mM) by Adipose Tissue *in vitro**

	CO_2	Glyceride-Glycerol	Fatty Acid	Glycogen
Glucose-U-C^{14}	2.540	2.660	0.810	0.090
Glycerol-1,3-C^{14}	0.126	0.371	0.018	0.003
Glycerol/Glucose	5%	14%	2%	3%

* Values expressed as micromoles substrate carbon per mg. tissue nitrogen per three hours.

epinephrine,[18] thus making untenable the hypothesis that epinephrine might inhibit the process of esterification. It was apparent, therefore, that epinephrine primarily accelerated the breakdown of triglyceride to fatty acids. Concomitantly, if there was an increased rate of glyceride-glycerol synthesis from glucose, a large portion of glycerol already present in tissue triglyceride prior to epinephrine stimulation and liberated by lipolysis was not re-utilized for the process of esterification. Determination of formaldehydogenic material in the medium after periodate oxidation with correction for reducing material present in the medium, showed that epinephrine did cause the release of a substance[27] which was subsequently identified as glycerol by paper chromatography.[28]

GLYCERIDE-GLYCEROL SYNTHESIS

Studies on adipose tissue by Shapiro, Chowers and Rose[29] suggested that "activated" glycerol (as reviewed by Wertheimer in this issue) in the form of glycerolphosphate, is required as the glycerol donor in the process of esterification and is similar to the precursor in the formation of triglyceride in liver[30] or intestinal mucosa.[31] Table I compares the incorporations of glucose and glycerol carbon into components of adipose tissue. It is evident that glycerol, compared with glucose, is a poor precursor of glyceride-glycerol, and is not well incorporated into CO_2, fatty acids and glycogen. Incubation of adipose tissue in the presence of epinephrine decreases the incorporation of labeled glycerol carbon into glyceride-

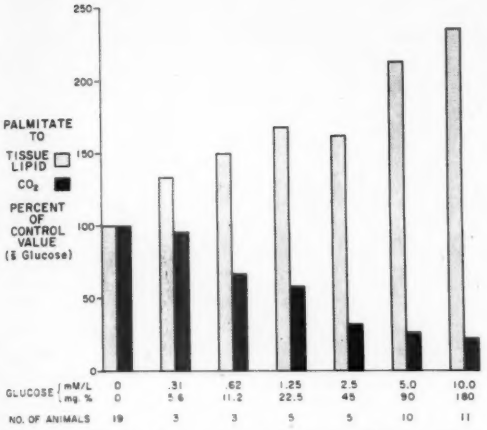

FIG. 3. Effect of glucose upon incorporation of palmitate-1-C^{14} into tissue lipids and upon its oxidation to CO_2 by rat adipose tissue. (From: Bally, P., Cahill, G. F., Jr., Leboeuf, B. and Renold, A. E. *J. Biol. Chem.*, 235: 333, 1960.[26])

glycerol by 33 per cent, probably due to a greater dilution of glycerol-C^{14} by unlabeled glycerol generated in the tissue.[28]

These results also suggest that the increased rate of re-esterification, as evidenced by accelerated glyceride-glycerol synthesis from glucose, is a secondary phenomenon. Further evidence for this conclusion was obtained by comparing glyceride-glycerol synthesis from glucose-C^{14} in tissues incubated at low (0.6 mEq. per L.) and high (3.5 mEq. per L.) medium fatty acid concentrations.[18] Incubation in the medium containing the high concentration of fatty acids resulted in increased glyceride-glycerol synthesis and the other associated changes in carbohydrate metabolism noted with epinephrine stimulation, namely, increased oxidation of carbon-6 of glucose and increased glucose utilization. The degree of these changes following the addition of fatty acid to the medium was less than those found in the presence of high concentrations of epinephrine, probably due to inability to elevate intracellular fatty acids to comparable levels.

CONTROLS OF RELEASE

The data therefore suggests that two factors control the rate of exchange of fatty acids between tissue triglyceride and medium, one being the esterification process, which is dependent on glucose for the synthesis of necessary glycerolphosphate, and the other the rate of lipolysis, which is sensitive to epinephrine. Other experiments have shown that growth hormone and adrenocorticotropic hormone preparations similarly increase the rate of lipolysis, thereby accelerating fatty acid release, increasing glucose uptake, glyceride-glycerol synthesis and glucose oxidation, particularly that of glucose-carbon-6.[32] In other words, these hormones produce a pattern of metabolism similar to that observed after epinephrine stimulation.

TABLE II

Glyceride-Glycerol Synthesis from Glucose-1-C^{14} and Glucose-6-C^{14} in Adipose Tissue *in vitro**

Glucose Concentrations μM	Insulin	Total Recovered Label†		Glyceride-Glycerol		Per Cent of Total Recovered Label in Glycerol	
		C-1	C-6	C-1	C-6	C-1	C-6
1.25	0	0.61	0.62	0.19	0.29	31	47
5	0	1.23	1.19	0.22	0.51	18	43
20	0	2.23	1.64	0.39	0.58	17	35
80	0	4.08	3.05	0.43	0.64	11	21
1.25	+	4.80	2.70	0.37	0.54	8	19
5	+	10.47	4.54	0.69	0.68	7	15
20	+	16.49	8.06	0.78	0.71	5	9
80	+	23.69	14.61	0.73	1.06	3	7

* Insulin 0.1 unit per ml. Values in micromoles glucose carbon per gm. adipose tissue per three hours. Values from JEANRENAUD, B. and RENOLD, A. E.[12]

† Sum of label in CO_2, glyceride-glycerol, fatty acids and glycogen.

TABLE III

Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} by Adipose tissue *in vitro* in Different Metabolic States*

	No. of Animals	CO_2		Glyceride-Glycerol		Fatty Acid		Glycogen	
		C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6
Fed + Insulin (0.1 unit/ml.)†	7	1.850 ± 0.218	0.225 ± 0.022	0.409 ± 0.033	0.584 ± 0.036	0.783 ± 0.138	1.482 ± 0.457	0.245 ± 0.037	0.264 ± 0.094
Fed†	38	0.353 ± 0.017	0.130 ± 0.005	0.323 ± 0.018	0.381 ± 0.012	0.056 ± 0.006	0.113 ± 0.016	0.013 ± 0.001	0.014 ± 0.001
Fasted twenty-four hours	3	0.153 ± 0.029	0.128 ± 0.017	0.237 ± 0.025	0.271 ± 0.019	0 015 ± 0.004	0.025 ± 0.005	0.008 ± 0.001	0.007 ± 0.001
Fasted seventy-two hours	3	0.090 ± 0.023	0.048 ± 0.007	0.166 ± 0.032	0.150 ± 0.020	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.004 ± 0.001
Alloxan-diabetic‡	4	0.026 ± 0.009		0.042 ± 0.020		0 ± 0		0.004 ± 0.001	

* Values in micromoles per mg. tissue nitrogen per three hours. Glucose 5 mM.
† Values from CAHILL, G. F., JR., LEBOEUF, B. and RENOLD, A. E.[11]
‡ Values for glucose-U-C^{14}.

TABLE IV

Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} by Adipose Tissue *in Vitro* in the Presence and Absence of Oxygen*

	Glucose Label	CO_2	Glyceride-Glycerol	Glycogen	Fatty Acids
O_2	C-1	1.064	0.435	0.017	0.238
	C-6	0.312	0.556	0.018	0.625
N_2	C-1	0.049	0.114	0.006	0.004
	C-6	0.028	0.082	0.007	0.009

* Krebs-Ringer-bicarbonate buffer gassed with 95 per cent O_2:5 per cent CO_2 or 95 per cent N_2:5 per cent CO_2 for ten minutes with the tissue present prior to the addition of labeled substrate. Glucose 5 mM. Values in micromoles substrate carbon per 350 mg. tissue per three hours. Mean of four tissues.

CONTROL OF ESTERIFICATION

Earlier in this report, glucose was described as accelerating the incorporation of palmitate-1-C^{14} into triglyceride. Other experiments have shown that adipose tissue from normally fed animals, is sensitive to glucose concentrations of 0.312 mM (5.6 mg. per cent) (Fig. 3). Thus a small amount of glucose is able to supply a significant amount of glycerolphosphate to effectively alter the fatty acid-triglyceride interchange in favor of esterification.

In Table II are tabulated the recoveries of glucose-C^{14} in total tissue components, in glyceride-glycerol and their per cent relationship. With low concentrations of glucose, in the absence of insulin, one-third or more of the glucose is recovered in glyceride-glycerol. As glucose uptake is increased by increasing concentrations of glucose, or to a greater degree, by addition of insulin to the medium, glyceride-glycerol synthesis becomes proportionately less important as a route for glucose metabolism.

In Table III are compared the metabolism of glucose-1-C^{14} and glucose-6-C^{14} in adipose tissue from fed rats, twenty-four and seventy-two hour fasted rats as well as the metabolism of glucose-U-C^{14} in adipose tissue from rats with alloxan-diabetes. As the metabolism of glucose becomes progressively less due to the fasted or diabetic state, a greater proportion is recovered in glyceride-glycerol. Likewise, incubation of adipose tissue in the absence of oxygen (Table IV) markedly reduces the total recovery of labeled glucose, with the least reduction in the synthesis of glyceride-glycerol.

GLYCERIDE-GLYCEROL *IN VIVO*

To determine whether the high rate of glyceride-glycerol turnover relative to the rate of

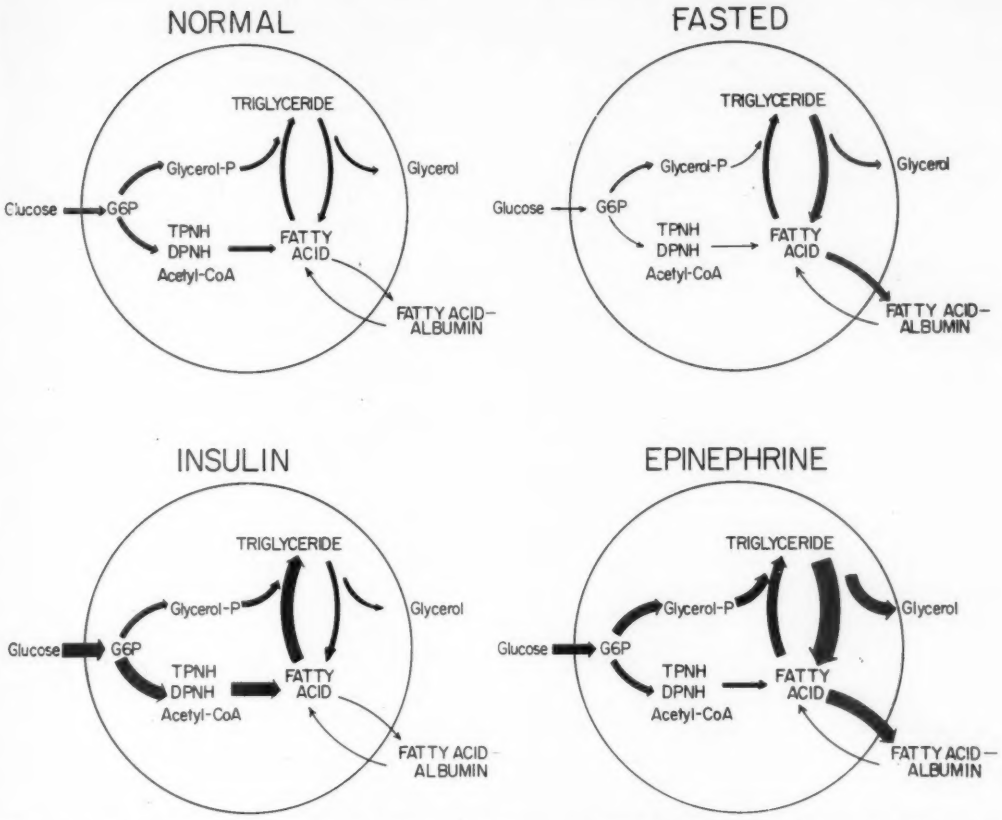

FIG. 4. Adipose tissue. A speculative summary of the concepts described in the text. In the normal rat glucose uptake is sufficient for the formation of glycerol-phosphate needed to esterify the fatty acids formed by lipolysis. In the fasted rat (or diabetic) glucose availability is decreased, consequently lipolysis outweighs esterification, fatty acids accumulate inside the cell and are released into the medium. With insulin, glucose uptake is much greater than that amount needed to esterify the fatty acids formed by lipolysis. The surplus glucose is converted to fatty acids and these also are esterified and deposited as triglyceride. After epinephrine, there is markedly accelerated rate of lipolysis, glucose uptake is increased as a response to the high concentration of fatty acids inside the cell, but the cell apparently does not re-esterify the major quantity of these fatty acids and they are released into the medium.

fatty acid synthesis was an *in vitro* artifact, uniformly labeled glucose-C^{14} was administered to rats which were killed one hour later. Table v summarizes data from these experiments and illustrates that twice as much glucose carbon may be recovered in glyceride-glycerol compared to the amount recovered in glyceride fatty acids. Since glycerol contains 3 carbons and the fatty acids in triglyceride an estimated 51 carbons, glyceride-glycerol is synthesized twenty to thirty times more rapidly than that amount needed to esterify the newly formed fatty acids.

COMMENTS

As glucose uptake is decreased in adipose tissue, whether by fasting, diabetes or anoxia, a greater proportion of glucose is recovered in glyceride-glycerol, suggesting that this pathway assumes first priority. Extrapolating from these observations to the total organism, a regulatory mechanism can be visualized whereby a decreased glucose uptake in adipose tissue allows the rate of lipolysis to exceed the rate of esterification due to lack of adequate glycerolphosphate formation. The smaller the glucose uptake, the greater the release of fatty

TABLE V

In Vivo Glyceride-Glycerol Synthesis from Glucose-U-C^{14}*

Rat	CPM/ 850 mg. Total Lipid	CPM/ mM Glycerol	CPM/ 3mM Fatty Acid	Recovery† of Counts (%)
A	6,000	4,770	2,190	116
B	10,500	3,990	5,130	88
C	5,400	3,930	1,730	105

* 125 gm. male rats injected intravenously with 55 mg. glucose containing 8 microcuries. Rats killed one hour later and epididymal fat excised and extracted.

† (CPM/μM glycerol + CPM/3μM fatty acid) ÷ CPM/850 mg. total lipid.

acids, in spite of the fact that the greatest proportion of the glucose is being converted to glycerolphosphate. As the glucose uptake is increased, whether by insulin or by increasing glucose concentrations, glycerol-phosphate synthesis increases until the rates of esterification and lipolysis are in a steady state, thereby inhibiting fatty acid release from the tissue. The surplus glucose is then metabolized by the adipose tissue for lipogenesis. These mechanisms are schematically illustrated in Figure 4.

Due to the exquisite sensitivity of this tissue to insulin, as measured by glucose metabolism, as perhaps occurs in mild or non-ketotic diabetes, only a small amount of insulin is sufficient to allow adequate glucose uptake, glycerolphosphate formation and triglyceride synthesis and thereby preventing the massive mobilization of fatty acids which would result eventually in ketoacidosis. On the other hand since the control of fatty acid release by the metabolism of glucose is presumably affected by chronic metabolic changes, the acute need for mobilization must be governed by increased lipolysis as suggested by the epinephrine studies *in vitro*[27] and discussed in this report.

SUMMARY

The rate of fatty acid release by adipose tissue is dependent on the relative rates of fatty acid esterification and lipolysis. Glucose is necessary for the esterification process, probably due to glycerolphosphate formation. Epinephrine and other hormones which accelerate fatty acid release appear to affect the rate of lipolysis. As glucose uptake is diminished, glyceride-glycerol formation becomes the major metabolic product of glucose utilization. As glucose uptake is increased, after glyceride-glycerol synthesis becomes sufficient to esterify the fatty acids, the surplus metabolized glucose is utilized for lipogenesis.

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Non-Esterified Fatty Acids in the Blood of Obese and Lean Subjects

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IT IS A common belief of physicians and laymen alike that basic differences in energy metabolism must exist between obese and thin human subjects. Despite the failure of biochemists and physiologists to identify any definite anomaly, the belief has persisted that a higher over-all thermodynamic efficiency of obese persons could explain their apparent low requirements of energy and the resistance to weight loss which becomes manifest when they are subjected to a sustained caloric deficit. The report herein deals with investigations bearing upon this problem as a result of the observation that the pattern of fat mobilization and utilization under fasting conditions is demonstrably different in fat and thin subjects.

Non-esterified fatty acids (NEFA) have been measured by the titration method described by Dole.[1] Venous blood is drawn at intervals throughout the day, using heparin as the anticoagulant. It is processed immediately by centrifugation and separation of the plasma, which is then extracted with heptane. Final titration with dilute sodium hydroxide is performed in a two-phase system agitated with a stream of nitrogen, using bromthymol blue as an indicator. All subjects are fasted throughout the test; the first blood sample is drawn at 8:00 A.M. after an overnight fast and the last specimen drawn between 4:00 P.M. and 5:00 P.M., so that the test period represents the last nine hours of a twenty-four hour fast. Quiet activity about the ward is allowed and drinking water may be taken as needed. Normal healthy subjects of average body build show a progressive rise in NEFA concentration in the blood during the test. The curves in Figure 1 represent the changes in four normal subjects, two men and two women. Values obtained in the early morning after the overnight fast are usually between 300 and 500 μEq./L. and rise during the ensuing nine hours to between 800 and 1,200 μEq./L. For unknown reasons, women tend to have somewhat higher values than men. Subjects, who are constitutionally thin, usually hyperkinetic and who are able to consume high caloric diets without any tendency to gain weight, show much steeper rises in blood NEFA values while fasting. Figure 2 illustrates values obtained for a twenty-five year old woman who is healthy, but literally unable to gain weight despite an excellent appetite. During four hours of fasting, the concentration of NEFA rose nearly 2,000 μEq./L. and then fell precipitously 1,500 μEq./L. when carbohydrate was ingested. These changes resemble somewhat the rise which accompanies strenuous physical exertion in normal subjects. The three curves shown in Figure 3 are from the same normal male subject: one, a control experiment; the second, illustrating the changes in concentration of NEFA induced by a diet very high in fat and the third, showing the extremely sharp rise brought about by vigorous exercise under fasting conditions. Obese subjects, on the other hand, show markedly elevated levels in the early morning which tend to remain essentially flat curves without any consistent trend upward or downward during the course of the standard test period. Figure 4 illustrates the typical flat curves in two fasted obese patients,

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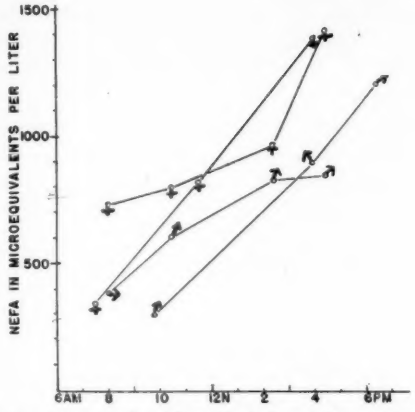

FIG. 1. Changes in NEFA levels in the blood, induced by fasting in normal subjects of average build.

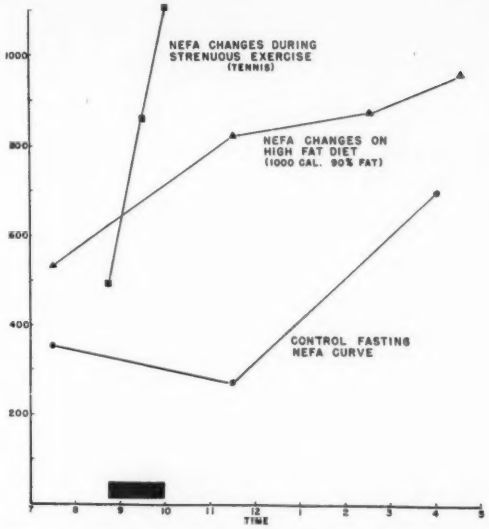

FIG. 3. The effects of strenuous physical exertion and a high (90 per cent) fat diet in a normal male subject.

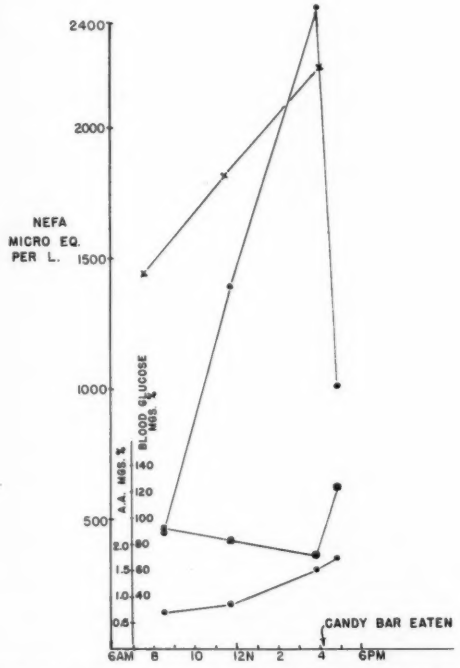

FIG. 2. Lipid changes in thin subjects during fasting: a twenty-five year old woman weighing ninety-five pounds; a twelve year old boy weighing forty-seven pounds.

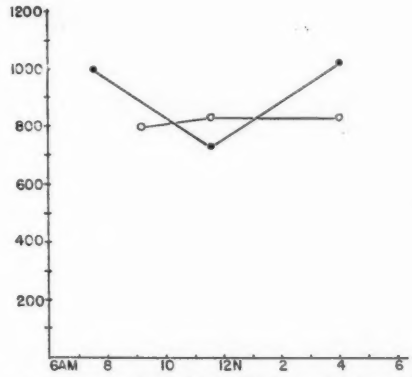

FIG. 4. Changes in lipid levels in marked resistant obesity during fasting. ● = twenty year old woman weighing 290 pounds. ○ = twenty-two year old man weighing 380 pounds.

a man and a woman. Figure 5 represents the changes in NEFA, blood ketones and glucose in two other obese subjects, both women. Here again, there is a conspicuous absence of the consistent, progressive rise seen in subjects of normal weight. Note the respiratory quotient of 0.78 in one of these women.

Certain obese subjects, who are clearly overweight because of constant excessive food intake, show NEFA curves that seem intermediate between the flat plateau and the usual steady rise. Such patients lose weight quite easily when caloric intake is restricted. Figure 6 shows, on the left, the NEFA curves of

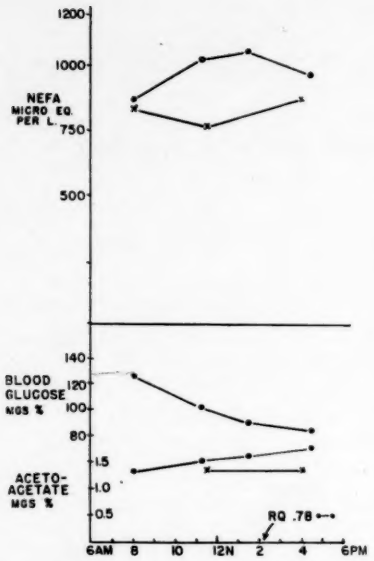

FIG. 5. Lipid changes in marked resistant obesity during fasting. ●—● = nineteen year old woman weighing 328 pounds; x—x = twenty-three year old woman weighing 215 pounds.

three patients, all of whom by history and response to diet were obese because of overeating. The curves show some tendency to rise, although the gradient is less than in normal subjects, and the values are all distinctly elevated. These women all lost weight easily when they were subjected to a sustained period of caloric restriction. On the right, curves from four "resistant obese" subjects show the characteristic flat curve at an elevated level; all had very small appetites, and none of these subjects lost weight even during observation in the hospital for prolonged periods of time.

Interpretation of these changes in concentration of NEFA, in the past, has been entirely in terms of changing rates of fatty acid mobilization from adipose stores, and the implication accordingly has been that fasted obese subjects do not mobilize fatty acids. Because of the dynamic nature of lipid metabolism, however, and especially because of the highly labile state of the NEFA fraction, it seems possible that the circulating level at any moment represents an algebraic summation of the rate of delivery from fat stores to the circulation and the rate of peripheral utilization of fatty acids for metabolic needs. Under these conditions, a rising level might be due to an accelerated rate of mobilization, a decreased rate of utilization, or both. Thus, a failure of consistent change in concentration of NEFA over a period of many hours, while it could be attributed to a failure of mobilization, might also be interpreted as a rapid rate of mobilization balanced by an equally rapid rate of utilization. Suggestive evidence that the latter might, in fact, be more correct was provided by the measurement of respiratory quotients which we, as well as others, have noted in obese subjects to be consistently below 0.8 and usually in the vicinity of 0.7. It is well known that such readings have classically been interpreted as indicative of predominantly fat oxidation.

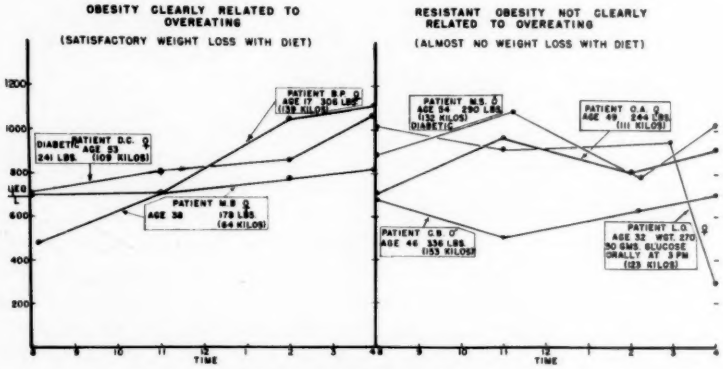

FIG. 6. Fasting blood NEFA curves in severe obesity.

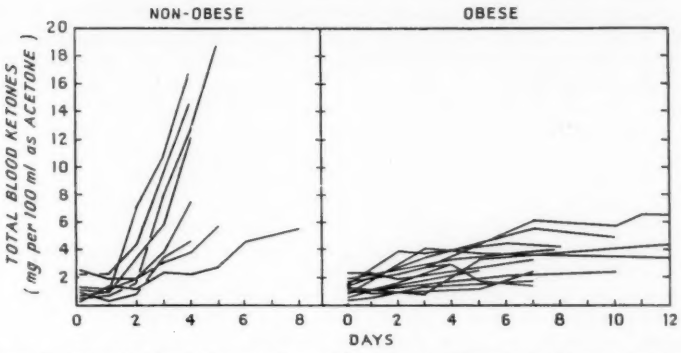

FIG. 7. Rise in blood "ketones" in subjects on a 1,000-calorie, 90 per cent fat diet. (From: KEKWICK, A., PAWAN, G. L. S. and CHALMERS, T. M. *Lancet*, 2: 1157, 1959.[2])

Thus it appears that obesity is characterized by an increased, rather than a decreased rate of fat utilization, as might initially be suggested by the experimental data. The resistance of fat people to the development of ketosis, however, seems difficult to reconcile with these other data. Figure 7 illustrates the sharp separation of obese from normal subjects in regard to the ketosis produced by a high fat diet.[2] We have repeatedly confirmed this finding and we believe that the phenomenon is probably due to some enzymatic mechanism in the liver in obesity which may protect Krebs cycle intermediates from the attrition that usually occurs in the absence of the constant regeneration normally derived from metabolism of carbohydrates.

In order to obtain further information on the dynamics of these changes in NEFA, studies have been instituted to measure the rate of oxidation of C^{14}-tagged palmitic acid administered intravenously to human subjects, both obese and normal. These have not proceeded far enough to report in detail, but it is already apparent that fatty acid degradation to carbon dioxide and water proceeds vigorously in obese subjects and even appears to be more intense and more rapid than in subjects with slim body build. Data of statistical significance have not yet been accumulated, but if the early trends are maintained, it would appear that obesity, at least of the resistant type, is characterized by a predominantly lipid oxidation for energy purposes in the course of ordinary metabolic needs. Such conclusions would correlate satisfactorily with the other indices of fatty acid oxidation, such as the constant low respiratory quotient in obese subjects.

Two additional clinical observations are worthy of mention. In those obese subjects with the flattest type of NEFA curve during periods of fasting, the sensation of hunger is consistently very slight or absent in contrast to the rather intense hunger of many or most of the slim subjects. This difference is apparent in both long and short fasting periods. It also seems probable from these data that the ease of weight loss, when a caloric restriction is imposed and maintained, correlates with the NEFA curve, so that those with the flattest curves lose weight with difficulty or not at all, and those with gradually rising or near normal curves (those subjects, with obvious obesity from habitual overeating), lose weight very easily under these conditions.

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DISCUSSION

DR. VINCENT P. DOLE (*New York, New York*):
I wonder whether Dr. Chalmers would care to ask any questions of his friends, and *vice versa*.

DR. CHALMERS: I would like to make a comment on Dr. Gordon's paper.

This characteristic of resistant obese patients in whom the NEFA levels do not rise with fasting, reminds us of patients we have observed and given ketogenic (usually 90 per cent fat, 1,000-calorie) diets. We find that it is extremely difficult to induce hypoglycemia and ketosis in very obese patients with diets of this kind.

I wondered whether perhaps there was some link in the mechanism for these two phenomena.

DR. GORDON: I am sure there is a link. We have had the same experience, but I have not been able to put it all together into a pattern.

Respiratory quotients at the end of a long fast in these fat people were constantly very low on the order of 0.75, 0.76, 0.77. This did not seem consonant with the hypothesis that they were not mobilizing fat because they were using some other fuel. If it were some other fuel, it must be carbohydrate. If they were using carbohydrate instead of NEFA, then there must surely be a rise in respiratory quotient. But there was none.

Therefore, it seemed to me that they were still living on fat, on their own fat and not on carbohydrate. What little evidence we have thus far would indicate that this is so. We are going to continue to experiment to discover if we can find out what the pattern of oxidation of fat is in these people.

We have two very bizarre observations on these resistant patients to whom we have given carbohydrate at the end of a long fast. Their respiratory quotient one hour later, instead of rising, fell further.

I do not think I have ever noted this before, and I have no explanation. It is almost as though the carbohydrate made them burn more fat.

DR. DOLE: Dr. Cahill, do you have any questions?

DR. CAHILL: No. I only want to comment on the extreme sensitivity of the mobilization effect of your factor, Dr. Chalmers. I think this is fascinating. It is down to 1 mg. per ml. of what still is probably a crude protein fraction. This is almost as active as ACTH and more active, certainly, than growth hormone. I think this is striking and very important.

DR. GORDON: Have you, by any chance, had occasion to examine the urine of patients who might fall into this category, who are obese and who are not able to lose weight?

DR. CHALMERS: Yes. I think, as far as we go, we do not detect any difference between the amounts obtained from obese patients and non-obese patients, but we have not really looked into this critically enough, because our assay methods are not very quantitative. I think this needs to be confirmed, but so far we do not really detect very much difference. We certainly get a great deal of activity in the urine of obese patients.

DR. DOLE: I now would invite general questions from the floor.

DR. LILLIAN RECANT (*St. Louis, Missouri*): I would like to ask a little bit more about this fat-mobilizing material in the urine. I wonder whether you have had any opportunity to look for any other metabolic effects of this material. For example, have you attempted to find out whether it has any of the patterns of growth hormone activity when injected into an animal? Does it produce growth? Has this been tested? Does it have any effects at all on, let us say, cardiac glycogen in the fasted animal? Is there any evidence of any of these activities? I understand that you have tested for the ACTH activity.

DR. CHALMERS: We have not studied cardiac glycogen, but it always causes loss of weight even in quite young animals when given for periods up to twenty-one days. So I think we can say it does not cause growth.

DR. JAMES M. SALTER (*Toronto, Ontario, Canada*): Apropos of Dr. Recant's question, one of the hypotheses, of course, has been that growth hormone acts by increasing the mobilization of fat and making more energy available for synthesis of protein and that the growth is an indirect outcome of this action.

I think the whole thing is rather interesting because of Dr. Chalmers' fat-mobilizing substance. Obviously there is an increase in the utilization of fat with no retention of nitrogen at all. And it seems there are a number of other things, like adrenalin and others and even vasopressin, which increase the utilization of fatty acids without any effect on nitrogen metabolism.

DR. CAHILL: I think this is true for all these factors. There is something unusual going on here. I mean, conservation of energy has to hold. In other words, if you have an animal that eats just a little bit less but still has a marked weight reduction, you cannot explain this just because NEFA moves out of adipose tissue and goes somewhere else in the body. Unless that energy is oxidized, some other process is going on.

The only possible explanation for the expenditure of energy has got to be in the basal metabolic rate. So I think you have to assume that these animals have an increased respiratory rate, just as do your glucagon-treated animals, Dr. Salter.

DR. GORDON: This phenomenon of people who do not lose weight is really the most tantalizing thing that confronts physicians. There are these people who can live on 600 calories and not lose any weight. On what are they surviving? If we measure their basal metabolism in terms of calories, we get figures in excess of 600 calories per twenty-four hours. It would seem that on this diet they are in a caloric deficit all the time, but still are not losing any weight.

I am still an admirer of the laws of thermodynamics, but these people seem to be thermodynamic paradoxes, and I certainly would like an explanation.

DR. DOLE: Does anybody have one?

DR. F. X. HAUSBERGER (*Philadelphia, Pennsylvania*): I would like to mention experiments which were made during World War I and II, especially during World War II, on obese people. There were certain types, who (when their food intake was very much restricted, say to 600 calories and even below) for quite a long time did

not lose weight, but then very suddenly lost weight. That weight loss then was water.

I think these people (whom you describe) on a diet of about 600 calories, retain some water, because there is no other possibility if, as Dr. Cahill pointed out, we believe in the laws of energy.

DR. GORDON: This point, supposedly, has been investigated by a measurement of total body water in these people, and it has been found to be normal or very slightly decreased. This is not our work, but it has been reported.

I think what we are all looking for, unquestionably, is something that controls efficiency. If any one of us in the room were to suddenly increase the efficiency of his machine by 1 per cent and all other factors in his life remained the same, he would immediately start to gain weight, but could accomplish the same amount of useful work with 1 per cent less expenditure of energy.

It does not take a large magnitude of change in efficiency to account for this sort of thing. But, to my knowledge, nobody has ever isolated any point in any metabolic scheme in which the efficiency might be changed, except for those instances like dinitrophenol or, possibly, thyroxin, where there is an uncoupling of P to O ratios.

DR. R. H. WILLIAMS (*Seattle, Washington*): Dr. Horth and I have carried out some studies a little like the one Dr. Gordon executed in regard to the fasting of these subjects. The NEFA levels of normal subjects, as you demonstrated, went up. The obese ones were somewhat like that, although, as you indicated, there were variations among different subjects.

I do not believe you made a comment, but I believe that your figures indicated that your obese subjects were also at a higher level than the normal early ones. This is something that puzzled me, and I have not quite understood that. It seems as though the obese subjects were failing to mobilize NEFA for some reason or under certain conditions they had more not being utilized.

The other point was that when we administered epinephrine, the values in normal subjects increased more than did the obese ones. Again, there was a defect in the mobilization.

DR. GORDON: Dr. Dole, I recall that you were the first one who showed that the obese subjects have a higher fasting level. Why is this? Do you have any ideas?

DR. DOLE: No, I do not. I thought, in the original interpretation, that there was some doubt as to how long the fat person had been functionally starved. At that time I had not thought of the possibility that the fat people might fail to rise, and it occurred to me that if I waited until, let us say, eight in the morning, after a night's dinner, to take blood out of a thin and a fat person, the fat person might be somewhat in the position that the thin person would be in at 10 A.M. I had thought that perhaps the time of starvation represented just a different functional interval for the two people. Now, of course, it comes into somewhat more question.

DR. MAX KLEIBER (*Davis, California*): I cannot get excited about the law of conservation of energy in this particular case, because so far I have failed to see an explanation of the necessity for the energy in basal metabolism for the vital functions.

Borsook made a calculation of the renal function, and he came to the conclusion that the kidney has a very high capacity for work with an extremely low efficiency. So the work of the kidney, certainly, cannot explain the basal metabolism. Neither can the work of the heart, nor the work of the muscles of the thorax.

I really do not see why you suspect that there is something wrong. Apparently, a good deal of the basal metabolism is just absolute waste. It is not a necessity for work, because the work of an animal can be rather highly efficient—it can be 25 per cent, 30 per cent efficient—and if he has that efficiency, then I do not see why an animal or a human being cannot exist on 600 calories instead of 2,000. Why should he have 2,000 calories unless he is in a cold environment? Then I could understand it. But that is another question.

Do you have those people tested in a cold environment, so that they give off that much heat?

DR. GORDON: If you are directing that at me, no, we have not. But I daresay that almost anyone in this room, if he subsisted on 600 calories per day, would very rapidly lose weight. I am sure I would. I don't know why, except that I always thought I was in a caloric deficit when I was getting that amount of food.

DR. DOLE: I think that perhaps some work we have done which has not been published might contribute a bit to that. It is a fact that when a person reduces by a caloric deficit, the metabolic rate does, indeed, decline very much. Obviously, the calories burned come out as heat, because the body temperature stays constant.

The question as to whether one is in caloric balance at 600 calories needs a little closer examination just in terms of method. You realize that if you expend only 10 gm. of fat per day, you get ninety calories. So a few hundred calories can be made up of what is almost a trifling amount of body weight, and that can easily be masked by a small amount of water or indeed just by the noise and inaccuracies in your weighing unless you have a much more accurate scale and a much more precise metabolic control.

The notion that people have controlled this by measuring body water is, I think, to some extent an illusion. You have 60 to 65 per cent body water. In a person who weighs eighty kilograms, there is a great amount of water and we are trying to detect the difference of a few grams. The methods, even if you give them 3 per cent or 5 per cent accuracy, are in the order of magnitude of a hundred times more uncertain than the small changes in water you are trying to reconcile. No measurements of body water are capable of discriminating between those little fluctuations that could replace a caloric equivalent of fat that would cover a deficit of a few hundred calories.

When one says that the fat person on a 600-calorie diet fails to lose much, if any, weight over a period of a month or six weeks, it still does not mean that the caloric expenditure is as low as 600. I think it might be 1,200 or 1,000. There is a marked decrease, in other words, but it need not be such an extremely low level as to be undetectable by the methods that are available.

DR. THEODORE B. VAN ITALLIE (*New York, New York*): I would like to raise a few questions that have troubled me in this discussion.

The assumption seems to have been made that the level of NEFA in the plasma somehow represents an indication of the rate of fat mobilization, which may be true under certain circumstances. But, after all, it is only a level, and fatty acids are not only being mobilized but also being disposed of. Certainly the level can be altered or changed by rate of utilization as well as rate of mobilization. If rate of utilization increases for some reason, there is no reason to suppose that the level would necessarily rise. Indeed, I sometimes wonder why it does rise at all.

The second thing that I wanted to bring up was the assumption that seems to have been made by several of the speakers today that in obese patients with diabetes there is a deficiency of insulin. From my conversations with Dr. Renold on his work with insulin-like assay procedures and with Dr. Mayer on his analyses of insulin in obese hyperglycemic mice, there is very little evidence that obese patients with diabetes are, indeed, deficient in insulin, at least as we ordinarily attempt to measure this substance.

The third point which is, perhaps, the most confusing, is the question of epinephrine. I believe Dr. Cahill said that epinephrine promoted the entrance of glucose into the adipose cell. I find this difficult to reconcile with some of the other data that have been given on the effect of insulin on uptake of glucose by rat diaphragm. Groen has demonstrated, I believe, that epinephrine is a powerful inhibitor of insulin activity as far as the rat diaphragm is concerned. Dr. Ingle has shown that in the eviscerated rat, epinephrine inhibits the rate of glucose disposal. Somogyi has shown that epinephrine impairs peripheral arteriovenous glucose differences. And the Drs. Cori, to mention another approach to this experiment, have shown that with perfusion of glucose through the hind limb of the dog, when epinephrine is given the rate of utilization of glucose is decreased. I wish that someone could clarify just what epinephrine does do in the periphery.

DR. CAHILL: You quoted the work of Dr. Ingle. The paper was by Ingle and Nezamis. On looking at the paper, he at times got an increased uptake of glucose in the extremity or in the eviscerated preparation. This was startling to him. He quoted this in his paper; occasionally there was no question but there was an increased uptake of glucose, and he also quoted some previous papers. This whole idea was reviewed by Griffiths in 1954 in *Physiological Reviews*—there are isolated experiments where it was found that epinephrine did, indeed, cause a glucose uptake.

Going over some of these experiments, these were experiments across a hind limb using, perhaps, the major vessels, which included the inflow of the saphenous. This makes one wonder whether epinephrine does cause a decreased glucose uptake in muscle and possibly an increased glucose uptake in adipose tissue.

One can offer a very brief explanation. The classic theory, of course, for the inhibition of glucose uptake by muscle is that in muscle, when you have glycogen breaking down to glucose-6-phosphate, glucose-6-phosphate being an inhibitor of hexokinase, the phosphorylation of glucose is inhibited. Parker and Kipnis have both demonstrated that when muscle is treated with epinephrine, there is an increased glucose concentration inside the cell but yet a decreased metabolism of glucose.

Now look at adipose tissue. Adipose tissue from the normal animal, as Dr. Tuerkischer and Dr. Wertheimer, I believe, demonstrated in 1942, has no glycogen, or a barely detectable amount. It is about 0.01 per cent as determined with the finest technics.

If epinephrine broke down this very small amount of glycogen, there certainly would be no inhibition of hexokinase by an accumulation of glucose-6-phosphate.

There is one other isolated discovery which was pointed out to me by Dr. Sutherland: if one increased the glycolytic rate in muscle by adding an oxidation-reduction mediator, in this case hydroxyquinoline; if one treated muscle with hydroxyquinoline and then administered epinephrine, that muscle exhibited an increased glucose uptake or increased glucose metabolism, which makes one wonder whether, on the cell wall, there is no epinephrine effect of inhibition. Once the possibility of glucose-6-phosphate inhibiting glucose phosphorylation is removed, epinephrine would then increase glucose uptake in muscle also.

DR. HERVEY (*Sheffield, England*): In relation to the obese person who eats very little, I should like to suggest that individual variations in energy expenditure should be considered. There are great variations, both in resting energy expenditure and in the energy required to do some defined task, such as walking at a particular speed. These variations are much greater than are sometimes realized when one looks at tables of normal metabolic rates.

Experience of the centuries has defined what is the normal food intake for a person of average energy expenditure: three meals a day with so many courses, and so on. If the person who happens to be low both in his needs for resting and activity is offered this, it is probable that if he eats what he is offered, he will tend to get fat.

There is a suggestion being made that there may be a feedback control of food intake, geared to the amount of fat in the body, the idea being that if excess fat accumulates this inhibits further food intake, so that the fat is held constantly and, incidentally, food intake matches expenditure.

If you had such a system, the effect would be that

as the person got fat as a result of his low need for energy, the accumulation of fat would gradually depress intake, and eventually balance would be reachieved when the depression was sufficient to offset the amount of food he was being offered. His intake would, then, be back to his low requirement The end result could be a person who was both fat and had a low requirement. In fact, the requirement might even be less than before because of the extra insulation, and the accumulation of fat would, in cybernetic terms, correspond to the load error of what I suppose we would call a lipostat rather than a thermostat.

Studies on Genetically Determined Metabolic Patterns

PAUL F. FENTON, PH.D.*

SOME TIME AGO we made the observation that under suitable conditions of maintenance the feeding of synthetic rations which are very high in fat could induce obesity in some strains of mice while leaving at least one strain lean.[1-3] In the most susceptible strains there is a linear relationship between dietary levels of fat and the amount of carcass fat deposition. In these susceptible strains one observes a sharp decrease in the rate of formation of the fat-free component while carcass fat increases linearly to five months of age. In the I-strain mouse the formation of both fat and fat-free components diminishes at two to three months of age. The result is a sharply reduced rate of weight gain and of the maintenance of lean carcass composition to at least six months of age. A variety of experiments have demonstrated that to a considerable extent the obesity induced by the feeding of a diet high in fat content can be accounted for on the basis of increased caloric intake with increase in caloric density of the diets. However, differences in activity[4] and in oxygen uptake[5] have been observed.

The existence of strains of mice differing so sharply from each other in their regulation of food intake led us to examine some of the underlying metabolic and endocrine patterns.

When A- and I-strains of mice were pair-fed a diet high in fat content[6] for a period of forty-eight days following weaning, more fat was deposited in mice of the A-strain than in those of the I-strain. This suggests the existence in the A-strain mouse of facilitated channeling into the fat depots, which may help to explain the failure of this strain to restrict food intake while on rations of high caloric density since rapid deposition of ingested fat may serve to reduce the intensity of the satiety signal.

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FAT MOBILIZATION

Another interesting aspect of fat metabolism has been the mobilization of fat to the liver during fasting.[7] The levels of fat in the liver in the fed state are the same for A- and I-strains of mice; however, large amounts of fat are mobilized to the liver in mice of the A-strain when they are subjected to a forty-eight-hour fast (Fig. 1). The I-strain mouse subjected to the same fast shows an almost imperceptible rise in levels of fat in the liver. This observation might be the result of slow mobilization of fat; it might also be the result of rapid oxidation of fat in the liver of the I-strain mouse. Since both the A- and I-strains of mice used in this experiment had been raised on a low-fat commercial ration, their body weights and fat content were about the same. Therefore, it cannot be argued that mice of the I-strain had less depot fat to mobilize.

In the experiment reported in Figure 1, forty-eight-hour fasts were initiated at different times during the day with different groups of animals. The increase in levels of fat in liver of mice of the I-strain between eight in the morning and two in the afternoon is statistically significant. It is probably due to a marked reduction of food intake of this strain during the daylight hours. The rise in levels of fat in the liver could be prevented by oral ad-

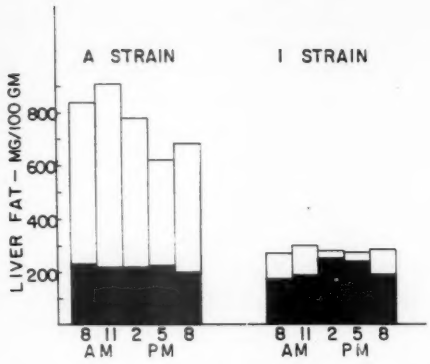

FIG. 1. Levels of liver fat (mg. fat/100 gm. initial body weight) of fed mice (solid bars) and forty-eight-hour fasted animals (open bars).

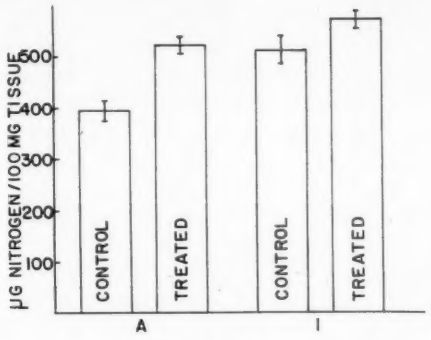

FIG. 2. Total nitrogen released by mouse diaphragm during three hours of incubation.

ministration (stomach tube) of a substantial dose of carbohydrate. The mice of the A-strain showed no such changes in levels of fat in the liver.

NITROGEN RELEASE

A variety of experiments demonstrated that turnover of protein in the I-strain mouse is significantly greater than it is in the other strains.[8] In a typical nitrogen balance experiment it was found that, at all levels of nitrogen intake studied, the nitrogen excretion of the I-strain mouse was significantly greater than that of the A-strain mouse. In a related experiment at the tissue level it was observed that the release of nitrogen, using the isolated diaphragm technic, was greater for tissues taken from animals of the I-strain (Fig. 2). This technic[9] involved measurement of the total amount of nitrogen released during three hours of incubation in a Krebs-Ringer solution.[10]

The greater turnover of protein in mice of the I-strain suggested that amino acids or other nitrogenous compounds might exist in large amounts in circulation and thereby contribute to the sensitivity of the regulation of food intake of these animals. However, the ultramicro methods required for repeated determinations of amino nitrogen and urea on 20 μl. quantities of tail blood have just been perfected. In the meantime an indirect approach to this problem was taken. On the supposition that increasing the level of dietary protein would increase circulating non-protein nitrogen, we increased the level of dietary protein from 30 per cent to 90 per in stages. This caused an irregular but marked decrease in the food intake of mice of the I-strain while comparable mice of the C3H strain were not affected.[6] Conversely, decreasing the level of dietary protein from 15 per cent caused a rise in food intake in all strains.

Efforts have been made to study certain aspects of carbohydrate metabolism. We have found no large differences in glucose tolerance, although the mice of the I-strain show somewhat better tolerance than the others. On the other hand, the levels of fasting blood sugar of the mice of the I-strain were consistently higher. Within each strain there was an increase in fasting blood sugar with increasing body weight.[11] The I-strain mouse showed extraordinarily high levels of muscle glycogen, but the levels of liver glycogen were lower than in the other strains.[12] *In vitro* studies showed isolated diaphragm and abdominal muscle of the mice of the I-strain to possess considerable glycogenolytic activity.

ENDOCRINE PATTERNS

Some tentative efforts have been made to modify the endocrine balance in the several strains of mice in the hope of throwing some light on the relative output of hormone. In the work on nitrogen release using the aforementioned isolated diaphragm we have compared not only tissues taken from normal untreated animals, but also those tissues taken

from mice injected with one of several hormones. In these experiments, at the dosages used, only thyroxine had a significant effect.[10] Daily treatment with large doses of thyroxine for seven days led to a large and significant increase in the release of nitrogen by the diaphragms of the mice of the A-strain. Similar treatment produced only a small and statistically insignificant increase in the release by I-strain tissue. It is tempting to suggest that these data indicate that the I-strain mouse operated normally at a relatively higher output of thyroxine than did the A-strain mouse.

CARDIAC GLYCOGEN

The mobilization of fat to the liver during fasting seemed so much more pronounced in mice of the A-strain that it suggested a greater output of the growth hormone in this strain. Efforts were made to utilize changes in cardiac glycogen during fasting or after administration* of the growth hormone as an index of the relative output of the growth hormone. Progress in this direction was hampered since neither fasting nor administration of the growth hormone caused any increases in cardiac glycogen; fasting for forty-eight or seventy-two hours actually caused a decrease. To test the effectiveness of our technics and the potency of our preparation of the growth hormone we repeated these experiments with rats and observed the changes in cardiac glycogen which was reported by Adrouny and Russell[13] and others. Similar increases in cardiac glycogen were obtained in the mouse only when the experiments were conducted at the temperature of 30°c. This rather elevated temperature maintains the mouse within a few degrees of its critical temperature of 33°c. At ordinary laboratory temperatures the rat also is close to its critical temperature of 27°c. Similar dependence on environmental temperature has been observed in some experiments on the role of adrenocortical steroids in protein catabolism. We have been able to show a significant drop in the adrenal ascorbic acid mouse when animals are moved from a room at 30°c. to one at 23°c. for a period of four hours. However, even at 30°c. the I-strain mouse showed no increase in cardiac glycogen during fasting, supporting the concept of relatively poor ability to release the growth hormone.

Many aspects of the nitrogen metabolism of mice of the I-strain suggest greater adrenocortical activity in this strain, however, some of our own observations are not compatible with this interpretation. Similarly, it is tempting to explain the high levels of muscle glycogen which occurred in mice of the I-strain on the basis of a greater output of insulin, but there are strong arguments against this. In order to define precisely the endocrine patterns of our four strains of mice, more direct measurements of hormone output must be made.

* The growth hormone used in these studies was a gift from the Endocrinology Study Section, National Institutes of Health.

SUMMARY

Genetically determined patterns of carbohydrate, fat and protein metabolism have been observed. To some extent these patterns have been related to genetically controlled rates of hormone output.

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DISCUSSION

DR. HERBERT S. ANKER (*Chicago, Illinois*): Have you crossed the mice of strains A and I? What happens?

DR. FENTON: We have done this. We did it some time ago when we were still engaged principally in nutritional studies. We applied certain very elementary criteria, such as nitrogen excretion on nitrogen-free rations to measure the endogenous nitrogen output, and the deposition of fat on high-fat diets to crosses and back-crosses of these strains. We discovered that the genetic factors which determine nitrogen metabolism and those which determine deposition of fat in the carcass are not the same. There may be some overlap, but the inheritance definitely does not coincide in the two systems.

DR. DWIGHT J. INGLE (*Chicago, Illinois*): In the 1930's an extensive series of studies was carried out on rats by Palmer and Kennedy at the University of Minnesota, who developed high- and low-efficiency strains by selective breeding. These studies were not well known because most of them were published in bulletins of the School of Agriculture. At that time, they did quite thorough studies, attempting to determine the reasons for the differences in efficiency of food utilization. However, the methods were not then adequate to reveal the reasons for the differences. I think that today our methods might give an answer why one strain can gain much more weight on a fixed amount of food than can another.

DR. FENTON: We have some measurement of physical activity in our strains of mice. On low fat rations, the mice of strain I are much more active, as might be anticipated. On high fat diets, the other strains tend gradually to increase activity while they are increasing their caloric intake. But, oddly enough, strain I reduces its activity in high fat feeding, which is very perplexing.

I hope, with the system we are now developing, that we can measure simultaneously activity and uptake of oxygen along with food consumption. I think all three need to be known.

Comments on the Genetics of Human Obesity

ARTHUR G. STEINBERG, PH.D.*

IN Gates's accumulation of the literature published prior to 1946,[1] I found numerous entries under the heading of obesity. With one exception these entries fell into two categories: (a) obesity associated with a known syndrome, such as the Froelich syndrome or the Laurence-Moon-Biedl-Bardet syndrome and (b) anecdotal, i.e., isolated pedigrees not critically examined. The single exception was Davenport's study[2] reported in 1923. Only one of the more recent reviews and texts, Kallmann's,[3] made reference to obesity. From his studies of twins Kallmann believes there is a correlation between obesity and manic depressive psychosis. He believes the latter has an important genetic component in its determination; therefore, so has the associated obesity. There is satisfactory evidence for genetic determination for only the Laurence-Moon-Biedl-Bardet syndrome. The data for the Laurence-Moon-Biedl-Bardet syndrome establish, if such is necessary, that genetic factors can influence obesity in man.

Davenport's pioneering study of the genetics of obesity in man has been largely ignored by geneticists as well as other investigators. True, the genetic analysis is naive in many respects, and Davenport chose to ignore important environmental effects which could have influenced the data. Nonetheless, he did recognize the problem and was aware of some of the possible ways in which the genotype could control tendencies to obesity. Thus, in his monograph Davenport wrote, "In general, it may be stated that variations in build are due to endogenous causes and exogenous causes. In this book we shall have occasion to examine especially the former—the constitutional or hereditary factors. These include idiosyncrasies of metabolism, in part controlled by peculiarities in the functioning of the endocrine glands; in part, probably, by even finer protoplasmic differences." Davenport then went on to enlarge on these items. He anticipated the modern somatotypists in attempting to find an association between body build and disease.

Davenport recognized that height and weight as such were not an adequate index of obesity and attempted to compensate for this by using an "index of body build" derived from height and weight, since these were the only figures he had. He found then that ratio, weight/stature^2 had the lowest variance and, therefore, used this ratio as his index. Only children at least eighteen years old were included in his study of families.

Davenport plotted the distribution of the weight stature^2 index (1,671 offspring of 506 matings). He arbitrarily divided the population into five groups—very slender, slender, medium, fleshy, and very fleshy. He then classified the parents and offspring in his sample according to these categories. Table 1 presents a summary of his data. We need not review his genetic discussion. The data do suggest a correlation between the parents' body index and the child's body index. Davenport indicated also that there is a correlation between the body index of husband and wife, confirming the popular belief that bodily contour does influence selection of a mate. What remains unclear from Davenport's study is why the correlation between parent and child exists. Is it entirely environmental, or is it at least in part due to genetic causes?

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TABLE I
Distribution of Progeny of the Various Matings According to Classes of Body Build

Type of Mating*	Total No. of Children	Proportional Frequencies (per 1,000)				
		VS	S	M	F	VF
VS x S	20	200	600	100	100	—
VS x M	28	36	250	607	107	—
VS x F	25	40	200	400	280	80
S x S	51	98	686	215	—	—
S x M	313	—	157	639	169	35
S x F	179	28	140	475	318	39
S x VF	50	—	140	360	340	160
M x M	332	6	121	605	247	21
M x F	346	—	90	606	255	49
M x VF	112	18	63	446	321	152
F x F	159	—	94	390	384	132
F x VF	146	7	48	356	349	240
VF x VF	37	—	—	400	267	333

* VS = Very slender; S = slender; M = medium; F = fleshy; VF = very fleshy. Data from: DAVENPORT, C. B. Publication No. 329. Washington, 1923, Carnegie Institution of Washington.[2]

Davenport believed that his analysis of individual families indicated a significant genetic component. I cannot agree with him because of his disregard of environmental factors which might have explained his data. So far as I have been able to determine, no one has accumulated data which can answer these questions satisfactorily. We remain convinced that obesity as such is influenced by genotype, but with no satisfactory objective evidence that this is so.

Our interest is to determine the nature of the genetic factor or factors leading to obesity and then to study how they affect the metabolic processes to produce obesity. Tepperman[4] outlined and illustrated by analogy various biochemical and physiological pathways by which the genotype might affect metabolism to lead to obesity or to leanness; I cannot improve on either the content or the form of presentation of this article. I can only state, in agreement with Dr. Tepperman, that the physiologic aspects of the genetic control of obesity cannot be solved until we know at least the general nature of the genetic pattern controlling these reactions. Only then will we be able to select the proper individuals to study.

Dr. Tepperman's paper and those presented at this symposium leave for me only the discussion of the complications associated with determining the pattern of inheritance. I have hinted at some of these when discussing Davenport's monograph. The simplest genetic problem concerns the study of a clearly defined easily identifiable character. Its appearance (phenotype) does not overlap that of its contrasting type, i.e., it is relatively insensitive to extra genetic influences. Furthermore, the phenotype is determined by one simple genetic mechanism only. Such characters are easily analyzed in experimental organisms, but even these are relatively difficult to analyze in man. For example, with the exception of the blood groups and certain serum factors, there is no single, genetically determined character in man which we can say with certainty is due to a change at one gene locus only. We cannot say with certainty that all cystic fibrosis or all phenylketonuria or all galactosemia is due to the same mutation. As a corollary I may add that, in general, the more variable the character, the less certain is our knowledge.

In regard to a more complicated phenotype, one which has a variable age at onset and a variable clinical expression, we find that geneticists cannot reach agreement about its mode of inheritance. Thus, several patterns of inheritance have been suggested for diabetes mellitus. Although more than a dozen extensive studies have been made of the genetic basis of this disease, sufficient doubt remains in the minds of some investigators to cause them to undertake new studies. In brief, genetic analyses in man are not simple and increase in complexity with depressing rapidity as the character studied grows more variable and more responsive to changes in the environment. Nonetheless, such analyses can be done, provided the data are carefully collected and properly analyzed and provided we recognize that the solutions for complex characters cannot be exact. New statistical procedures now being developed for analyzing data for such characters offer hope that many more problems

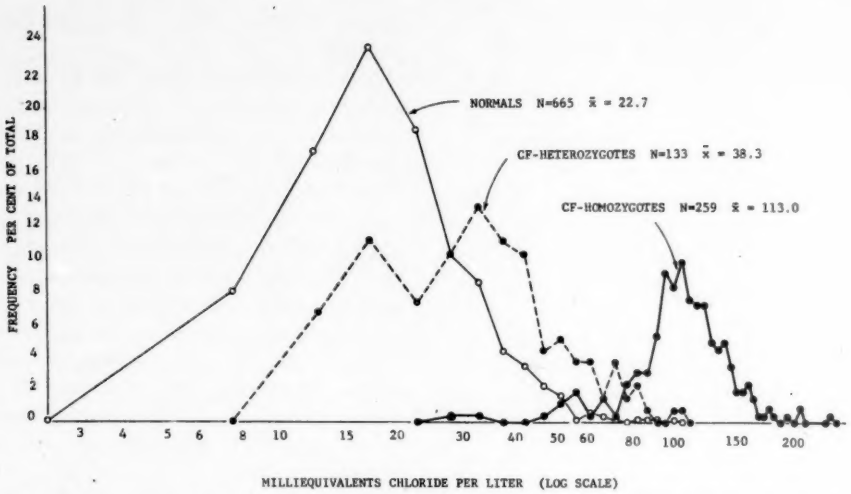

FIG. 1. Percentage distribution of chlorides in mEq./L. in the sweat of 665 individual subjects not known to be related to patients with cystic fibrosis, 133 parents of patients with cystic fibrosis and 250 patients with cystic fibrosis. Data from: SCHWACHMAN, H., STEINBERG, A. G., DOOLEY, R. and STERN, M. Unpublished data.

in human genetics will be solved with greater effectiveness.

Can we profitably study the genetics of obesity in man? I think we can. I propose to outline some possible approaches. Obesity is usually defined as an excessive accumulation of fat, and for our purposes this accumulation should not be the secondary result of another disorder. This definition would exclude obesity due to the Cushing syndrome, the Froelich syndrome, the Laurence-Moon-Biedl-Bardet syndrome, etc. We have now to define the word excessive. According to the second edition to the New Gould Medical Dictionary, excessive means the "accumulation of fat, beyond 10 to 20 per cent of the normal range for the particular age, sex and height." I present this definition only to indicate that we are not dealing with an all-or-none phenomenon; that we are dealing with a continuous variable and that it had best be dealt with as such. Unfortunately such phenomena are the most difficult to analyze genetically.

The difficulty stems from the fact that the continuously varying factor may be due to a series of genes with additive or multiplicative effect or both, or to sensitivity to environmental factors, or to some combination of these.

Some illustrations from human genetics are cystic fibrosis (Fig. 1), phenylketonuria (Fig. 2), for single gene differences yielding continuous variables, and serum cholesterol level (Fig. 3) and blood pressure as variables dependent upon age, sex, various environmental factors and possibly several genes as examples of more complicated forms.

The methods used to solve these problems, i.e., problems of continuous variables, with experimental organisms; establishing inbred lines, selection, control of environment, control of matings and so on, are not available in human genetics. Nevertheless, solutions are available. One practice is to select, among affected individuals, those who are twins and then to investigate the status of the co-twin with respect to the character in question. On a genetic basis one would expect the identical co-twins to be affected more often than the fraternal co-twins. Furthermore, the fraternal co-twins should be affected no more often than ordinary sibs. One would expect that characteristics which are particularly susceptible to psychological influences could not be effectively studied by this method, because identical twins would be apt to have more similar psychological experiences than fra-

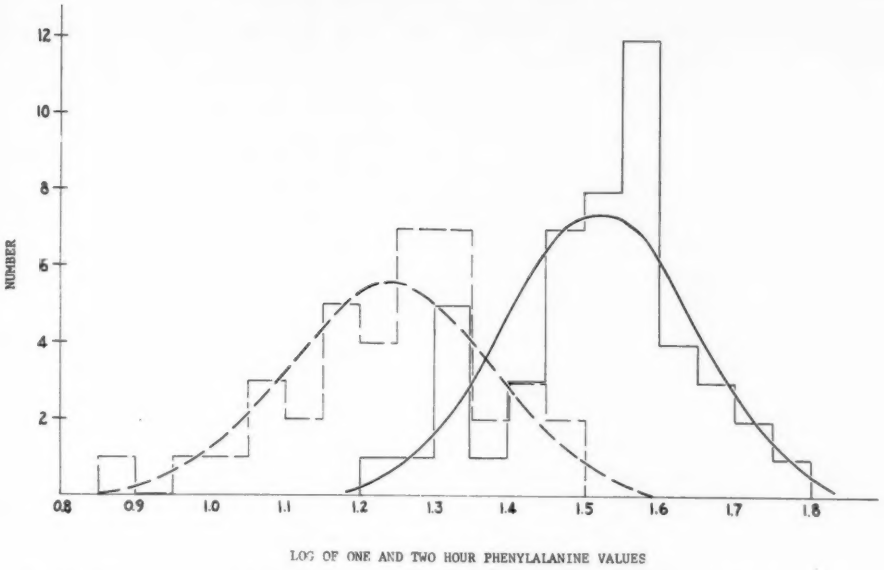

FIG. 2. Histograms and fitted normal curves for the distribution of the sums of the levels of L-phenylalanine in the serum of thirty-eight normal adult subjects (dashed lines) and forty-eight heterozygotes (solid lines) 1 and 2 hours after oral administration of $^1/_{10}$ gm. of L-phenylalanine per kg. of body weight. Data from: HSIA, D. Y.-Y. and STEINBERG, A. G. Studies on linkage between phenylketonuria and the blood groups. *Am. J. Human Genet.*, 12: 277, 1960.

ternal twins, who in turn would have more similar experiences than ordinary sibs. Nevertheless, several investigators have found that the frequency of schizophrenia among the fraternal twins of twin index cases is 10 to 15 per cent, i.e., no more frequent than among ordinary sibs of these patients, while the frequency among identical twins is 85 to 90 per cent. This technic could be applied to the problem of obesity. As I indicated earlier it would perhaps be best to treat obesity as a continuous variable and to compare the correlation coefficient for the measure of obesity between identical twins with that between fraternal twins, and with that between sibs. A measure of the fat layer such as skin fold or roentgenogram of the soft tissue would be desirable, to avoid the necessity for introducing corrections for height and body build. Only like-sexed pairs would be used, of course. Difficulty in interpreting such data would probably arise from our lack of knowledge of how great an influence various acquired habits have on weight. If it were noted that identical twins were more alike than fraternal twins and that fraternal twins were no more alike than sibs we would have strong evidence in favor of a genetic component in the causation of obesity. We could then select identical twin pairs, only one of whom was obese, and study the other

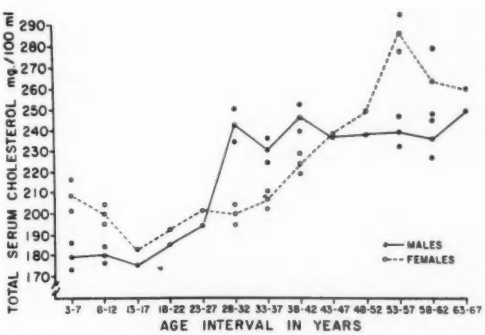

FIG. 3. Total serum cholesterol levels by age in men and women. (From: ADLERSBERG, D., SCHAEFER. L. D., STEINBERG, A. G. and WANG, C. Age, sex, serum lipids and coronary atherosclerosis. *J.A.M.A.*, 162: 620, 1956.)

TABLE II
Correlation Coefficients* of Serum Cholesterol Levels

Relationship	N†	r‡	P§
Mother-father	201	0.0056	>.2
Father-child	373	0.2101	<.001
Mother-child	373	0.3646	<.001
Sibling-sibling‖	123	0.3701	<.001
Father-son	181	0.1558	<.01
Father-daughter	192	0.2616	<.001
Mother-son	181	0.3402	<.001
Mother-daughter	192	0.3903	<.001

* All cholesterol levels converted to equivalent levels for men, aged 20, to eliminate factors of age and sex.
† N = number of pairs
‡ r = correlation coefficient
§ P = probability that the sample is from a population with r = o.
‖ Intraclass r based on first and second siblings only.
Data from: SCHAEFER, L. E., ADLERSBERG, D. and STEINBERG, A. G. *Circulation*, 17: 537, 1958.

in the ways described by Dr. Tepperman and members of this symposium.

Another approach used in human genetics for continuously variable characters dependent upon age and sex is to introduce a correction which will translate all values to one equivalent to that for a given age and sex, and to compute correlation coefficients between the parents, the parents and the offspring, and between the offspring. According to genetic theory under the simplest assumptions, and assuming random mating, the correlation coefficients should be as follows: parent-parent, 0; parent-child, 0.5; sib-sib, 0.5. Deviations from the basic assumptions can lead to changes in these values, but if there is a strong genetic component concerned in the determination of the character, the order of the correlation coefficients should be as indicated above. As a practical indication, consider the following data for serum cholesterol level. The level varies with age and sex (Fig. 3). By smoothing the curves, it is possible to compute coefficients of regression for various portions of them and to use these regressions to compute corrections to convert all values to equivalent values for men, age twenty, for example. Using these corrected values, we can compute correlation coefficients. These are shown in Table II. For serum cholesterol level as for

TABLE III
Hypercholesteremia in 201 Randomly Selected Families

No. of Parents with Hypercholesteremia	No. of Families	Children		
		Total	Hypercholesteremic	
			No.	%
1	19	36	6	17
0	182	337	8	2
Total	201	373	14	4

P <.01

No. of Children with Hypercholesteremia	No. of Families	Parents		
		Total	Hypercholesteremic	
			No.	%
1 or more	13	26	6	23
0	188	376	13	3
Total	201	402	19	5

P <.001

Data from: SCHAEFER, L. E., ADLERSBERG, D. and STEINBERG, A. G. Heredity, environment, and serum cholesterol. *Circulation*, 17: 537, 1958.

obesity, one can choose an arbitrary point and say that all those who exceed the point are hypercholesteremic. If the upper 5 per cent level is chosen, as it has been in some studies, we again find evidence for a genetic component in the determination of serum cholesterol level (Table III).

Can we apply these methods to the study of the genetics of obesity in man, knowing that the parent-parent correlation is not zero? I think we can if we keep in mind both parts of the statement—that the necessary and sufficient criterion to establish a significant genetic component in the determination of a character is an increased familial incidence of the character *in the absence of environmental factors which can explain the increased incidence.* The last clause poses the difficulty confronting us in

a study of obesity. There are a myriad of environmental factors which may explain a familial association of obesity. Despite this, studies of twins combined with studies of sibs could supply valid data for determining the presence of a genetic factor in the etiology of obesity. More information could be gained be appropriate family studies, although we may still not learn how many loci are involved in causing the obesity. It would probably not be profitable to start familial studies on an ordinary population because of the difficulties in evaluating and controlling environmental factors which could cause familial similarity in obesity. Ideally we require a population in which the environment is essentially uniform from family to family; a population in which the eating habits do not differ from family to family, in which the housing, clothing, working, social, and educational factors are essentially uniform from family to family. At first this sounds like an impossible order for families living in the western world; but there is a population of about 11,000 people located in the United States and Canada which fulfills these requirements. There are about 110 colonies in all, located in South Dakota and Montana in the United States and in Manitoba, Alberta, and Saskatchewan in Canada.

The population is a sect of Anabaptist protestants who live a communal life based on Acts 2:44–45, of the Bible "And all that believed were together, and had all things common; And sold their possessions and goods, and parted them to all *men*, as every man had need." They live in colonies of approximately one hundred people per colony on a farm of about 5,000 acres. The men run the farm using modern farming equipment and technics. Each man has a particular task, i.e., care of chickens, pigs, cattle, grain, machinery, etc. The women do the cooking, cleaning, gardening and child rearing. All clothes are bought communally, and all dress alike regardless of their assigned tasks. Each family has its own suite of rooms, but the suite does not include a kitchen or a dining room. All food is prepared in a communal kitchen and eaten in a communal dining room. Usually there are two dining rooms, one for the young children, and one for the older children and the adults. Each dining room has two long tables; men eat at one table, women at the other. Large platters or bowls containing the food are placed on the table, and each individual helps himself. Hence, each person in a colony is offered the same food, and no one has had an opportunity to learn his eating habits from his parents or other related adults.

As part of medico-genetic study, we are collecting data on height, weight, and skinfold thickness of these people with a view toward determining the presence of a genetic component in obesity.

I had hoped to have some data to present, but unfortunately, we had not accumulated enough in time for it to be processed for this symposium. The study has not progressed very far and can easily be modified to introduce improvements.

On *a priori* grounds as well as on the information supplied by Davenport's study, we must expect that the genetic component in determining susceptibility to obesity is complex and that superimposed on this complexity are complications introduced by extragenic factors which influence the weight of an individual. These complications combined with difficulty in defining obesity have discouraged geneticists from studying the problem. I think it highly unlikely that genetic technics presently available will do more than permit us to identify families in which there is a great probability of genetic susceptibility to obesity. The biochemist and physiologist in studying these families may discover a basic metabolic difference which in turn would help the geneticist to define the problem more precisely for his studies and he in turn may then be able to select appropriate individual subjects for metabolic studies.

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DISCUSSION

DR. JEAN MAYER (*Boston, Massachusetts*): We have been collecting reports of twins. At the present time I think we have about 150 pair of non-identical twins and about ten or twelve pair of identical twins, whom we hope to follow both in regard to obesity and the incidence of dental caries. This is a joint study with the Forsythe Dental Infirmary.

Meanwhile, I think it might be useful for those of us who are interested in prevention and the public health aspect of obesity, to remember that while there is no good method at present for distinguishing between the environmental and genetic components in familial obesity, this is a very striking fact. In the communities of Brookline and Newton, Massachusetts we find that, on the average, about 8 per cent of children of non-obese patients are obese; if one parent is obese, the proportion is about 40 per cent; if both parents are obese, the proportion is of the order of 80 per cent.

Because everybody agrees that prevention of obesity is much easier than the cure, I think it is unfortunate that the very strong familial incidence of obesity has not been stressed more in public health programs, when particular attention should be given to children of obese parents.

DR. BARBARA MOULTON (*Washington, D.C.*): I have seen a study on twins (I think it was in the German literature) in which they compared fraternal twins and identical twins, and those who had been reared together and separately. My recollection is that the data revealed that there was considerably more variation in the weights of fraternal twins raised in the same household than in the weights of identical twins reared in separate households.

DR. JAY TEPPERMAN (*Syracuse, New York*): There was one study by Camerer and Schleicher some years ago. Is that the one you are referring to?

DR. MOULTON: I don't remember.

DR. TEPPERMAN: They had some interesting pictures of the superimposable silhouettes of identical twins.

DR. STEINBERG: The only study of that nature of which I am aware was done at the University of Chicago. This study was performed on nineteen pairs of identical twins who were separated. They were compared with fifty pairs of identical twins who grew up in the same household, and fifty pairs of fraternal twins.

I had not mentioned that study because there were only nineteen pairs and the portion of the study on obesity was somewhat casual. There was some indication that the identical twins who had been separated were more alike than the fraternal twins who were raised in the same household, again suggesting the involvement of a strong genetic component.

DR. VINCENT P. DOLE (*New York, New York*): In relation to this question of what to measure, what to look for, one feature that interests me very much is the physical activity. As you know from various studies, particularly that of Dr. Mayer's group published in the *American Journal of Clinical Nutrition* a couple of years ago, and Stunkard and others, there is a correlation between obesity and physical activity. Which is cause and which is effect? Do people of a given occupation tend to be slim because they work hard, or is the selection of the occupation in part determined as an expression of the physical vigor of the person?

I would suppose that in the present study, even though these people are involved in similar occupations, there is probably quite a range of physical demands. I do not know what indices you might construct or what pedometers you might use to measure relative activity in this community, but it would be interesting to know. Perhaps in this case you might come closer to the answer than in almost any other situation, in trying to look for cause and effect of physical size and occupation.

DR. STEINBERG: We are recording each person's occupation on the farm, but we do not have any measure of the actual activity. We shall be able to make a comparison of all goose-feeders versus those who work out in the grain fields, but we shall not know whether among goose-feeders there is variation.

There is some differentiation in the amount of activity in that the colony preacher generally works less vigorously than the others, although he is not simply a preacher. He does do physical work and carries on the trade he had before becoming a preacher.

DR. TEPPERMAN: Dr. Joliffe reminded me that in the famous epidemiologic study done in England by J. N. Morris on the incidence of coronary disease in bus drivers and conductors on London buses, in which the uniform sizes that were issued to the men when they started work were examined, they discovered that the girth of the people who elected to drive buses was larger at the time of onset of work than the girth of the conductors at the same time. What you say about job selection may be correct.

DR. WILLIAM PARSON (*Charlottesville, Virginia*): Do people leave the colony? Is there selection in this?

DR. STEINBERG: Yes, there is some selection in this sense. Women do not leave the colony.

They had, in the past, lost between 1 and 2 per cent of the adult men. This is not an accurate figure, for two reasons. The original number is not too accurate and a fair number of those who leave come back.

Since the war, however, their rate of loss has increased, but I do not have figures to quote. The rate is being increased because of the draft. These men now go to camps for conscientious objectors, where they live for two years and learn our way of life and become more independent.

You see, they have been raised on communal farms, where they have never handled money, never bought

anything, never asked for a job, never really taken care of themselves the way we have to. Leaving the colony is quite a change. When they have been out and trained in a camp for conscientious objectors it is different.

DR. PARSON: Are you going to study the psychologic attitudes toward obesity in these people?

DR. STEINBERG: I am not equipped to do so.

The young girls, I can tell you, are as conscious of obesity as our young girls are.

DR. MAYER: Amplifying what Dr. Parson said, I think there is very little doubt that the obese children for the most part tend to be very inactive. We believe that the only way to get to the root of the question that Dr. Dole raised is to start with the very young children and do both psychologic attitude tests and psychomotor endurance tests, because it is by no means certain which came first. The only way to find out is to begin testing at a very early age, since inactivity tends to produce clumsiness and is to be self-perpetuating among children who don't like to do what they do not do well.

Obesity and Cancer Susceptibility in Mice

SAMUEL H. WAXLER, PH.D., M.D.*

IN 1949 Brecher and Waxler were first to report the experimental production of obesity in mice induced by a single injection of aurothioglucose. Mice so treated developed varying degrees of true obesity. Weights of 60 to 80 gm. in males and 50 to 60 gm. in females were observed[1] (Figs. 1A, B and 2). Autopsies of these animals and chemical analyses of total body lipids, proteins, water and ash indicated that the gain in weight were primarily due to an increase in adipose tissue.[1] Some increase in the weights of the organs of obese animals was also apparent in the wet, dry and defatted states.[2]

During the period of development and maintenance of obesity the *ad libitum* consumption of food of "gold-treated" mice exceeded that of the control animals (Fig. 3). However, this obesity was readily regulated since such animals given a diet identical in caloric value to that of the control mice maintained the same weight levels as those of the control mice.[3]

Obese animals were able to mobilize their excess fat and withstand starvation for a period of from thirteen to seventeen days, while the control mice were able to endure starvation for about five to six days only. Subsequent feeding again produced obesity at the prestarvation level in animals starved down to their original weight. This indicated that the hypothalamic "lesion factor" induced by gold thioglucose was a permament one.[3]

The relationship of tumorigenesis to diet has been investigated by many workers during the past several years. The results of overfeeding and underfeeding experimental animals have generally indicated that diet has some effect on the development of both spontaneous and experimentally induced neoplasia.[4–10] Experimental evidence has been produced to show that a diet with a high fat content tends to promote or enhance the formation of many types of tumors, whereas a restricted food intake induces a delay in such formations. However, in most of these experiments comparisons were made between animals fed restricted or modified diet and those with unrestricted food intake. From such experiments we believed that no definite conclusions could be drawn as to the effect of obesity on tumor formation because the animals could not be justifiably termed truly obese. In our experiments true obesity was readily induced in the animals by an injection of gold thioglucose, and it was subsequently eliminated or reproduced by regulating the animal's food intake. These specific characteristics gave us a tool by which we could study the effects of obesity on the occurrence of spontaneously developing tumors in various strains of mice.

We produced evidence that spontaneous mammary tumors appeared earlier and in greater numbers in virgin mice of the C_3H strain made obese by an injection of gold thioglucose than did tumors in control mice of normal weights. The average age of the obese mice at tumor onset was 242 days compared to 303 days in the control animals (Fig. 4). Similarly, early occurrence of tumors was apparent in mice made previously pregnant as well as in the virgin mice. Gold-treated animals which did not become obese had the same incidence and rate of tumor appearance as did untreated mice.[11] Subsequently, we demonstrated that mice made obese and later reduced and maintained at the weight level of the control animals by paired feeding showed a slower rate of tumor

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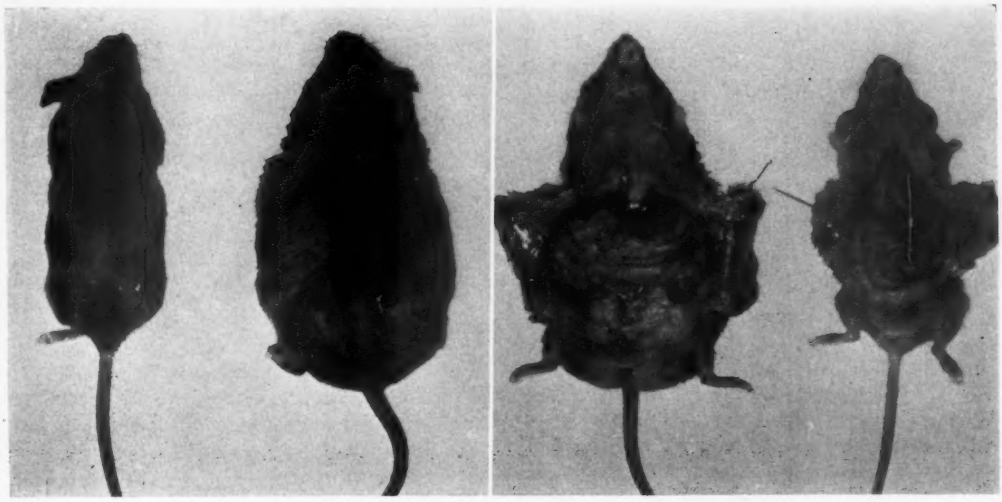

A B

FIG. 1. A, control C_3H mouse (37 gm.) and obese C_3H mouse (68 gm.). B, the same animals as in A, opened to show viscera.

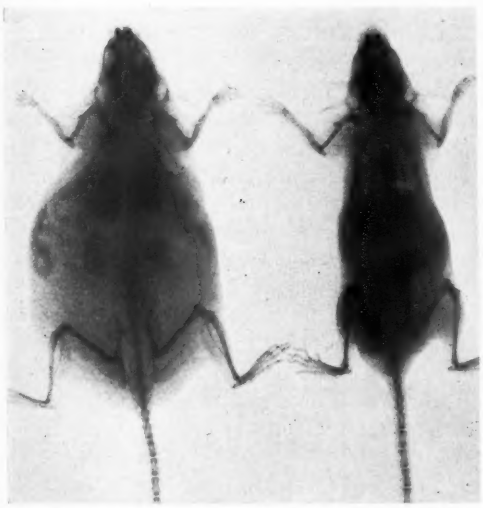

FIG. 2. Roentgenogram of obese and control mice.

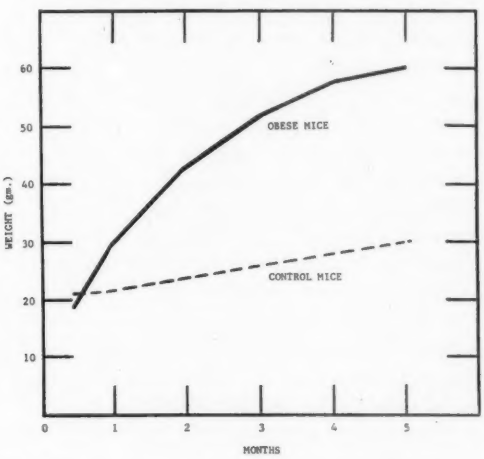

FIG. 3. Average weight gains of "gold-treated" and control mice over a five-month period.

incidence than did the control mice.[12] In this particular experiment the average number of days before tumor appearance in 50 per cent of the obese mice was 300 days, in the control mice 367 days and in the previously obese mice more than 400 days. Tumor incidence in previously obese animals was significantly less than that in their pair-fed partners, and much less than that in the obese group. In fact, after nearly all the control and obese mice were dead, a high percentage of reduced animals were still alive and tumor free.

It is well established that the appearance of mammary tumors in C_3H mice is associated with the presence of a mammary tumor agent or milk factor.[13] In control groups of C_3H mice lacking this agent, which we denoted as the C_3H-IICrgl mice, no mammary tumors ap-

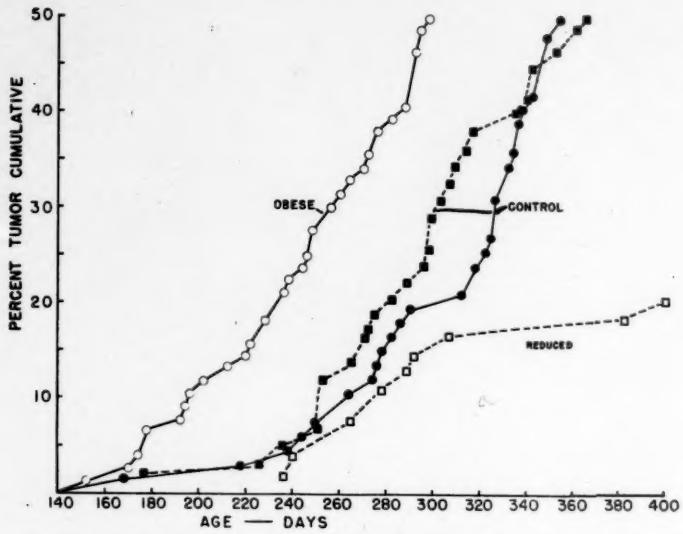

FIG. 4. Occurrence of spontaneous mammary tumors in obese, control and reduced (previously obese) virgin female C_3H mice.

peared during a period of twelve months. Similarly, during this same period C_3H-IICrgl mice made experimentally obese developed no mammary tumors. It was apparent that obesity and associated caloric intake do not incite the production of mammary tumors in animals lacking the specific tumor agent (Table 1). When the factor for tumorigenesis is present, the incidence can be increased or decreased by multiple factors from either within or without the animal. When the factor is missing, obesity or increased caloric intake, which otherwise accelerates or augments tumor appearance and incidence, has no effect at all.[14]

It has been demonstrated that tumor incidence in female C_3H mice is markedly reduced following gonadectomy. We believed that a study of the augmenting effect of obesity on tumor incidence as opposed to the depressing effect of castration would be valuable. A comparison of the tumor production of obese mice and mice of normal weights which had been castrated at the age of two months, with uncastrated normal and obese mice yielded typical results: the obese mice developed the greatest number of tumors or 46 per cent; the control animals, 31 per cent; the castrated obese mice, 14 per cent; and no tumors developed in the castrated mice of normal weights. Their ages at the onset of mammary tumors averaged 270

TABLE I

Incidence of Spontaneous Mammary Carcinoma in Female C_3H and C_3H-IICrgl Mice

Group of Mice	No. of Animals	Average Weight (gm.)	No. of Tumors	Per Cent of Tumors	Average Age at Tumor Onset (Days)
C_3H-IICrgl control	32	30	0	0	..
C_3H-IICrgl obese	30	52	0	0	..
C_3H control	23	32	8	35	333
C_3H obese	21	54	12	62	252

TABLE II

Effect of Castration on the Onset of Spontaneous Mammary Tumors in Female Obese C_3H Mice

Group of Mice	No. of Animals	Average Weight (gm.)	No. of Tumors	Per Cent of Tumors	Average Age at Tumor Onset (Days)
Control	35	32	11	31	298
Control-castrated	41	34	0	..	..
Obese	41	50	19	46	270
Obese-castrated	49	49	7	14	317

TABLE III

Incidence of Hepatomas in Obese, Control and Pair-Fed (Previously Obese) Male C_3H Mice

Group of Mice	No. of Animals	Average Weight (gm.)			Hepatomas	
		Initial	4 Weeks	13 Months	No. of Tumors	Per Cent of Tumors
Control	22	26	28	36	2	9.9
Obese	9	26	42	48	5	55.5
Pair-Fed	24	26	42	35	4	16.6

days in the obese mice, 298 days in the control animals and 317 days in the castrated obese mice. When this experiment was concluded at the end of one year, no tumors had appeared in the castrated control animals (Table II). The number of tumors which developed in the obese castrated group of mice was under the influence of two factors: (1) the removal of ovaries suppressed the appearance of tumors generally found in obese and control animals; (2) the increased caloric intake, obesity *per se*, augmented tumor development, and thus partially negated the influence of castration.

Continuing in a similar line of investigation we were able to show that spontaneously developing hepatomas increased in male C_3H mice made obese by administration of gold thioglucose. In a typical experiment, 64 per cent of the obese mice developed hepatomas; whereas, only 28 per cent of the contro[1] mice developed primary tumors of the liver.[15] The incidence of hepatomas in mice which were made obese and subsequently reduced to the weight of the control mice by paired feeding was close to that of the control animals. These reduced mice,

TABLE IV

Incidence of Spontaneously Developing Hepatomas in Control, Obese and "Multiple Injected" (Cumulative) Male C_3H Mice

Group of Mice	No. of Animals	Average Weight (gm.)	Hepatomas	
			No. of Tumors	Per Cent of Tumors
Control	32	33	5	16
Obese	31	47	14	45
Cumulative	29	32	1	3.4

TABLE V

Incidence of Spontaneous Hepatomas in Control and Obese, Male and Female, C_3H-IICrgl Mice (Lacking Tumor Agent) at the End of Twelve Months

Group of Mice	No. of Animals	Average Weight (gm.)	No. of Tumors	Per Cent of Tumors
Male control	36	32	4	11
Male obese	29	50	10	34
Female control	32	30	1	3.1
Female obese	24	49	12	50

therefore, did not develop the great increase of tumor incidence evident in obese mice which were allowed to eat *ad libitum*. It was apparent that having the potential of obesity did not lead to an unnatural occurrence of tumors (Table III). Only persistent obesity over a sufficient period of time was of importance.

In all experiments, male animals which did not become obese following injection with a toxic dose of gold did not show an increase in incidence of liver tumors. Apparently the mere presence of gold itself was of no consequence. It was noted that a large amount of gold in the liver, induced by multiple small intraperitoneal injections of gold thioglucose (the total of which is many times greater than the single toxic dose) was not a factor in the increased incidence of hepatomas. A single dose sufficient to produce obesity resulted in increased incidence of neoplasia (Table IV).

Generally, it has been difficult to ascertain the number of hepatomas in female C_3H mice for usually death due to mammary tumors occurs and the animals do not survive long enough to give a true incidence of the hepatomas which would occur.[16] The use of C_3H-IICrgl mice lacking the mammary tumor factor circumvented this obstacle. The appearance of a single hepatoma in our female group at the end of one year agrees with literature wherein the reported incidence of hepatomas in females is small and much lower than in males. However, when the females were allowed to become obese there was a definite increase in the occurrence of liver tumors. At the end of twelve months 50 per cent of our female mice had such tumors (Table V).

We have observed no pregnancies in the truly fat C_3H mice during the ten years of producing obesity by the administration of gold thioglucose. In an effort to study the factors associated with this observation we paired female C_3H mice, which were four months old, with normal males; within the next month all of the females were pregnant. After delivery and weaning, the mice were put into three groups: one group in which the parous mice received a single dose of 8 mg. of gold thioglucose; one in which the mice were given multiple small doses for thirty days; and one group in which the mice remained untreated. Six weeks later the animals of these groups were again mated with normal males. At that time the obese mice averaged 48 gm. in weight, the mice giving multiple doses weighed an average of 29 gm. and the average weight of the control group was 31 gm. No pregnancies appeared in the group of obese mice while all the animals in the "multiple injected" and control groups became pregnant. Subsequently, the obese mice, starved down by paired feeding to the weight of the control animals (i.e., about 32 to 34 gm.), were again allowed contact with males. No pregnancies appeared in this group of previously-obese reduced mice.

As a continuation of our work concerning pregnancies in obese animals, smears of C_3H virgin female mice were studied over a period of time. After a set pattern of estrus cycle was determined, a certain number of these animals were made obese. A change in the cytology of the vagina was apparent in subsequent smears of these obese animals. In most of the animals long periods of cornification of cells had occurred. When such animals were mated with males known to be capable of impregnation, no offspring were produced. None of the animals which became what we considered truly obese produced offspring; however, litters were produced by those gold-treated animals which did not become obese.

The ovaries from obese mice, in which long periods of cornification of cells had occurred, were observed (macro- and microscopically) and found to be atrophic. They consisted of follicles and only a few old corpora lutea. This was indicative of a continuous estrogenic phase, which may have accounted for the augmentation of at least the mammary tumors in obese mice.

SUMMARY

In summary, we have shown that mice can be made obese by injection of gold thioglucose. Animals so treated develop lesions in the hypothalamus and subsequently become obese. Animals made obese by this technic show two interesting phenomena: such mice have an augmentation of tumor production and truly obese animals do not become pregnant.

Hormonal imbalance occurs when hypothalamic obesity is produced. A condition results which apparently allows sufficient estrogen production to induce an increase in tumor production and at the same time keeps these obese animals from going through a normal estrus cycle.

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DISCUSSION

DR. JEAN MAYER (*Boston, Massachusetts*): Without wanting to detract from the results that have been presented here, I think that we should guard against the tendency to generalize results unduly. Dr. Waxler has not done it. But I think there would be a temptation to conclude that there is a promotion of tumors by a high caloric intake.

If we look at a table of mortality in relation to body weight in man we see that there are a great many diseases in which mortality is increased by obesity: diabetes, heart disease, et cetera. There are two causes of death which are decreased: tuberculosis and suicide. The deaths from tumors are the same in obese persons as in non-obese persons.

I do not think that this means that there is no interaction between caloric intake and tumors, but I think that it may depend on the type of tumors and the type of obesity.

A few years ago we demonstrated that in the obese hyperglycemic syndrome there is an increased resistance to liver ascites tumors, which is an unusual type of tumor, and an unusual type of obesity. I think this illustrates the fact that the interaction may be extremely complex.

One point I was interested in was the fact that, Dr. Waxler and his co-workers have had no luck in obese pregnant gold thioglucose animals, which we have observed and which have always been observed. There is a paper by Hamilton in this subject, which reported a number of obese pregnant animals. And there was also a French paper a few years ago, I have forgotten the authors, which described a number of obese gold thioglucose animals which became pregnant.

I was wondering whether the duration of the obesity might be a factor in the ability of these animals to become pregnant.

DR. WAXLER: No, I do not think that is the factor We have studied the reactions of these obese mice, in various strains, for about twelve years. An instillation of sperm can be performed, but this does not result in pregnancy. We have used young and old males, and young and old females in these experiments.

We thought there might be even physiologic difference between these animals, but we have not proven this. The animals which we used were genetically pure, and usually produce the first time they are mated. This pattern of reproduction ceases when they are made obese. Smears indicate that they are sterile. If the animals have a continuous estrogenic phase, with cornification of cells, and they do not show any corpora lutea and if what one sees are the old corpora lutea which may have been there before the mice were injected, then I cannot see how pregnancy could be induced.

DR. JAY TEPPERMAN (*Syracuse, New York*): I agree with Dr. Mayer that you really cannot learn very much by looking at over-all cancer statistics in obesity.

Some years ago, stimulated by Dr. Waxler's publications, we investigated some of the clinical reports on the incidence of cancer in obese patients. We noted that there was some indication in the clinical literature that the incidence of both cancer of the breast and endometrial cancer concurred with obesity whereas other types of cancer did not. There was a clear-cut association between the incidence of obesity and incidence of cancer in these two types of cancer—the same two types studied by Dr. Waxler.

DR. C. N. H. LONG (*New Haven, Connecticut*): We also noted the failure of the reproductive function in the females of these animals. There was a reduction in the size of the ovary in the rats which we studied. At that time, we were inclined to associate this with the development of obesity. Now, however, I believe that these failures are probably due to damage to other areas of the hypothalamus that are concerned with the release of the ovulation hormone.

DR. Waxler, did you observe the testicular function in the obese male mice?

DR. WAXLER: No, we did not.

DR. B. K. ANAND (*New Delhi, India*): I agree with Dr. Long's remarks. If the obesity has been produced by giving gold thioglucose, there will be damage to large areas in the hypothalamus. From experimental work, it is evident that the regulation of the gonadotropic function from the hypothalamus, at least in albino rats, is in the area surrounding the vendomedial nucleus. If some other means of producing obesity is used, these disturbances in the hormonal patterns may not be evident. But if you use large doses of gold thioglucose and damage this area extensively, not only the gonadotropin-secreting activity but other hormonal patterns will be upset.

Therefore, is it the simple increase in food intake which influences the production of cancer or of these tumors? Is it the disturbances of all these hormonal patterns in these animals? If this problem is studied

again without producing damage to this area of the hypothalamus, but by producing obesity by force feeding or giving high caloric diets, it will be interesting to see if there is a change in the tumors then.

DR. WAXLER: There is an increase in tumors in those animals that have been confined in very small areas. They do not get quite as large as the animals that are given gold. Of course, these confined animals are able to reproduce.

DR. TEPPERMAN: When you were studying hepatomas in restricted-feeding situations in animals with hypothalamic lesions, there seemed to be a slight suggestion that in those animals the incidence of hepatomas was slightly greater than in the control group. I wonder whether those animals ate their food all in one gulp, whether they were really meal-eaters, as Dr. Cohn has taught us.

DR. WAXLER: I do not know.

DR. ESTELLE R. RAMEY (*Washington, D. C.*): Dr. Anand's suggestion is ruled out by Dr. Waxler's experiments in which he fasted the animals with similarly large lesions. In those instances one would anticipate the same degree of endocrine anomalies, and yet those animals, in some instances, had fewer tumors.

Overfeeding as a Stress

GORDON C. KENNEDY, M.B., PH.D.

STRESS is a simple enough idea, either in ordinary life or in physics, but it has acquired almost mystical overtones in physiology. The title of this paper is not meant to imply that the adrenal cortex indulges in any esoteric ritual in response to overfeeding. It suggests indeed, that metabolic adaptation to hyperphagia leads to structural as well as functional changes in a variety of tissues,[1] and that these initially beneficial adaptations may eventually, after a period of time, result in pathologic lesions.[2] In much the same way, the initial elasticity of many physical materials under stress may finally give way to fatigue as may be illustrated from the structural effects of overfeeding on the liver.

ADAPTIVE CHANGES IN THE LIVER

When hyperphagia is induced in rats by hypothalamic lesions the food intake doubles overnight. In twenty-four to forty-eight hours the weight of the liver may increase by the same proportion. This increase in the weight liver does not involve a significant alteration in the percentage of water in the tissue, instead the solids of the liver double in amount. The earliest change is a great increase in glycogen, which for twenty-four hours or so may contribute more than half of the dry weight of the organ. Next the fat content rises, and as more protein is synthesized, the percentage composition of the liver gradually becomes normal again.[1] Studies on nucleic acid in these livers have shown that for the first three weeks the liver cells increase in size without dividing, but after six weeks there is an increase in the number of cells. The process is similar to the normal growth of the liver in a young rat. In the obese animal the rest of the body is not growing in the true sense, but simply getting fat.

These structural changes reflect successful functional adaptation to overfeeding. Significant impairment of glucose tolerance in the early stages of hyperphagia has not been found, The studies by Dr. Mayer's group which showed that, in spite of the greatly increased intake of carbohydrate, the hyperphagic rat maintains a lower concentration of blood sugar in the fed state than normal.[2] Dr. Lukens mentioned the studies of Ogilvie which showed the same type of results were seen in patients.[3] Dr. Tepperman has emphasized the role of adaptive hyperlipogenesis in the changeover from a low to a high plane of nutrition, and Dr. Siperstein has shown how increased use of the hexosemonophosphate shunt, such as occurs during adaptation, may lead also to increased protein synthesis.[5]

All these are changes which probably make increased demands for insulin, as first suggested by Dr. Brobeck and his colleagues.[6] Direct assay of blood insulin, as is expected, is not rewarding in this situation, presumably because the hormone is utilized rapidly. However, hyperphagic rats tolerate relatively large amounts of injected insulin (40 to 50 units a day of zinc protamine insulin) without ill effect although this is twenty times as much as a normal rat will tolerate. The insulin is not bound in the over-abundant fat, because starving for even one night, which has no appreciable effect on the fat content, restores the insulin sensitivity to normal.

It is of interest that Osborn, Felts and Chaikoff[7] showed that an almost identical sequence of changes in liver composition to those previously described in the hyperphagic rat took place in the liver of animals with alloxan dia-

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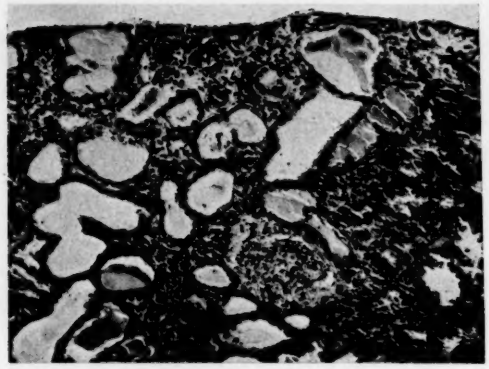

FIG. 1. Dilated cortical tubules and hyalined glomeruli from a "senile" rat aged two years. (H&E ×120.)

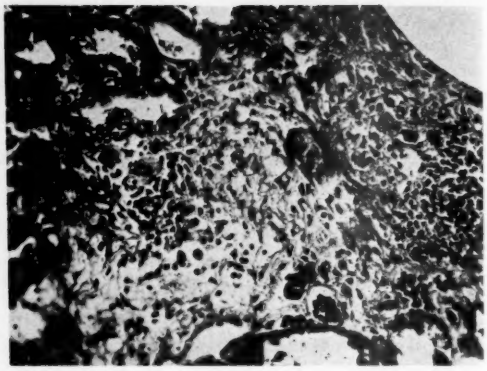

FIG. 2. Cortical scar in kidney of a two-year old rat. (H&E ×250.)

betes at the beginning of treatment with insulin. Moreover, in a number of studies on the effect of experimental endocrine abnormalities upon the changes in the liver in hypothalamic hyperphagia, alloxan diabetes was the only condition in which the adaptive liver growth did not occur. Adaptive growth can continue in the absence of the pituitary,[8] but apparently not in the absence of insulin. This seems to be a basis for regarding insulin as the growth hormone for the liver.

After a number of months, the glucose tolerance of obese animals frequently deteriorates, as demonstrated by Dr. Brobeck and his colleagues. In this late stage, the liver, although greatly enlarged, becomes fatty and its protein content is less than would be expected from its size. It is reasonable to suggest that the continued demand on the pancreas for an excessive secretion of insulin finally leads to its exhaustion, although significant abnormalities in the islet cells have not been seen with ordinary histologic methods.

KIDNEY DISEASE IN THE OBESE ANIMAL

Actuarial statistics have made us familiar with the fact that chronic kidney disease, like many other degenerative diseases, is more commonly found in obese subjects than in people of normal weight. It was of considerable interest, therefore, when Brobeck, Tepperman and Long[9] reported that obese rats killed six to nine months after hypothalamic operation had advanced kidney disease, with heavy proteinuria. They called the lesions chronic glomerulonephritis, although during microscopic study they appeared more like healed pyelonephritis, and all the lesions seen in this study in obese animals have been of pyelonephritic type. Most people who have worked with the kidney of the rat are familiar with this type of lesion because it frequently occurs in senile animals.[10] The main difference between the fat animals and ordinary stock rats was that kidney disease developed in the fat animals soon after they were a year old, while the general stock rats remained healthy until they were about two years of age. Although, the histologic appearance is that of so-called chronic pyelonephritis, the etiology is as obscure as that of the same lesion in man.

One of the difficulties about assigning any specific cause to the condition is the large variety of ways of which one can induce the disease in adult rats. In fact, almost any trauma applied to the kidney of a young growing rat tends, after a latent period of some months, to result in the premature appearance of these "senile" lesions of the kidney. Acute choline deficiency damages the kidneys of weanling rats, but animals which survive the original treatment may, in addition, develop chronic kidney disease months later along with hypertension.[11] Again, a long latent period is characteristic between irradiation of the kidney and the development of hypertensive kidney disease.[12] Seyle[13] has

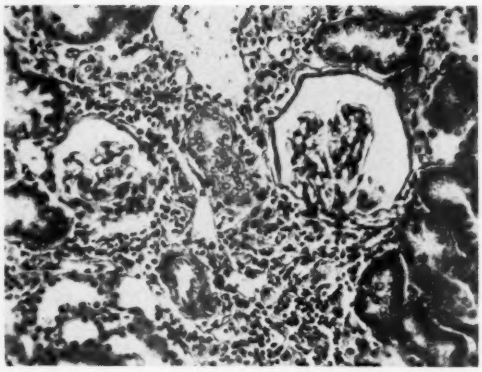

FIG. 3. Atrophic glomeruli and hyperplastic tubular epithelium in a rat aged two years. (H&E ×250.)

shown that a short course of injections of cortexone acetate, or of a crude pituitary extract, may produce little apparent ill effect at the time, but chronic kidney disease will follow months afterwards. There are many other examples of this long latency between the primary injury to the kidney and the development of hypertensive kidney disease.

It is interesting how similar the published photomicrographs of all these chronic lesions appear, and how closely they resemble the non-specific lesions of old age. Figures 1 to 3 illustrate this type of lesion in a two-year old rat.

Figure 1 shows an area of renal cortex with prominent dilated, cast filled distal tubules, many of which are replaced by scar tissue as shown in Figure 2. In Figure 3, two ischaemic, hyalinized glomeruli can be seen, and between them a tubule filled to the point of obstruction with hyperplasia epithelial cells.

ADAPTIVE GROWTH OF THE KIDNEY

The easiest way to produce premature lesions of the kidney is to remove one kidney from a healthy young rat. Nothing could be more complete and normal than the immediate recovery of such rats, however, the long-term survival of such animals is poor. Chronic kidney disease usually develops within the rats at about the same age as in the obese rats. It is interesting, therefore, that the initial effect of unilateral nephrectomy on the adaptive growth of the surviving kidney closely resembles that of overfeeding. As can be seen from Table I, the effect of a normal intake of food in an animal with only one kidney is the same as that of doubling the intake of a rat with two kidneys. It appears that the whole key to the mechanism of the final renal breakdown lies in the overloading of healthy tissue.

What about the period of latency before these healthy nephrons break down? Here the true

TABLE I

Composition of the Kidneys of Obese Rats and Rats with One Kidney Removed at Three to Six Weeks After Operation

Time from Operation	Group	Total Nitrogen (mg. per kidney)	Ribonucleic Acid Phosphorus (mg. per kidney)	Desoxyribose Nucleic Acid Phosphorus (mg. per kidney)
	Control*	19.4	0.378	0.183
Three weeks	Kidney removed	27.8	0.504	0.210
	obese	29.9	0.488	0.227
Six weeks	Kidney removed	29.4	0.516	0.289
	obese	28.3	0.532	0.308
Twelve months†	Control	34.9	0.749	0.239
	Kidney removed	73.5	1.429	0.695
	obese	75.9	1.972	0.785

* Rats upon which operations were not performed.

† Rats upon which operations were performed at three months of age.

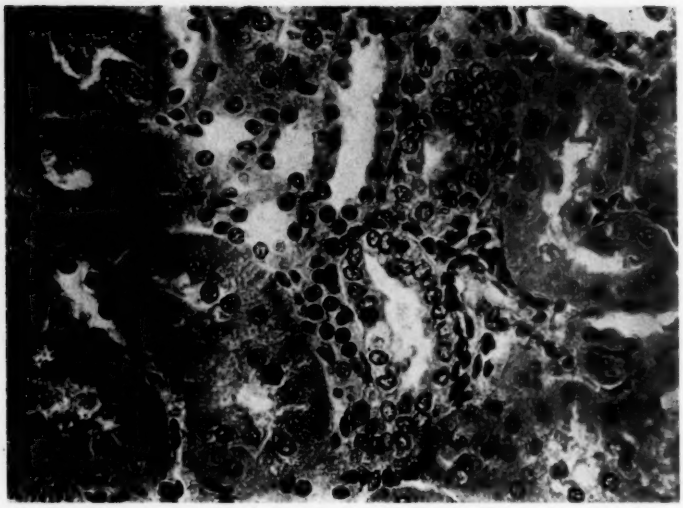

FIG. 4. Hyperplastic tubular epithelium in a rat aged two years which had not yet developed any other signs of renal pathology. (H&E ×420.)

aging of the kidney must be taken into account, not the final pathologic lesions of senility but something which begins much earlier in life, and is seen in man as well as the rat. This amounts to a slow atrophy and disappearance of nephrons, and judging from glomerular counts, it begins when the rat is about six months old and increases in rate with advancing age.[14] It has been shown that this process goes on regardless of renal hypertrophy from other causes.[15] Heminephrectomy will cause the remaining kidney to grow larger, but it will not arrest, or even slow down, the rate of atrophy of nephrons caused by aging. The older a rate becomes, the more the extent of any surgical or traumatic removal of kidney tissue, or the effect of any other form of renal overloading, will be increased by the further loss of nephrons due to aging.

PATHOLOGIC OVERGROWTH IN THE KIDNEY

There is a limit to the load that a given amount of kidney tissue can withstand. As this limit is approached, the kidney tissue fails to hypertrophy normally under load, but undergoes pathologic changes instead.[16] When less than the critical load is given to a young rat, the animal survives until the further loss of nephrons, due to aging, reduces its available kidney to the critical level. When this stage is reached, the kidney rapidly increases in weight, and pathologic changes take place. Much of the increase in size is caused by the dilated tubules.

It can be shown chemically that there is a considerable increase in dry weight and protein, and, of particular interest, a rise in nucleic acid content of the kidney (Fig. 1). The structural analogue of this increase in nucleic acids is the tubular hyperplasia which is seen in Figure 4, the first pathologic change observed in the "senile" kidney. This begins in the proximal convoluted tubule, but soon spreads to involve the whole nephron, and is probably the cause of the subsequent dilatation of tubules (Fig. 1).

TUBULAR HYPERPLASIA IN THE AGEING KIDNEY

Hyperplasia of this occurs in many forms of chronic kidney disease. It is usually regarded as the natural consequence of the loss of some nephrons due to the disease, causing compensatory overgrowth in others. However, this is an inadequate explanation, since the usual result of partial nephrectomy in an adult rat is hypertrophy of the remaining tissue rather than hyperplasia. The capacity to form new tubular cells as a compensatory phenomenon

appears to be lost quite early in life.[17] One must look for some extra stimulus to explain the sudden occurrence of hyperplasia in the tubules of senile kidneys. The most likely stimulus is overactivity of some of the endocrine glands, notably the adrenal and the parathyroids. There are a number of reasons for making this suggestion. Let us consider them in relation to the adrenal cortex, first.

Both in the senile rat, and in the obese rat undergoing premature renal breakdown, the adrenal is hypertrophied. A number of workers have shown that, even in the young rat, extensive removal of kidney tissue is followed by an increased production of urea, which seems to be associated with increased gluconeogenesis in the liver as well as with excess glucocorticoid. If an organ which normally forms a target for the secretions of an endocrine gland is removed, the gland tends to overact, and a feed-back of this sort has been proposed to explain the secondary aldosteronism of some forms of renal disease. Mineralocorticoid hormones certainly act as potent stimuli to hyperplasia in the kidney of the rat. Recently, it has been found that the sensitivity of the rat kidney to damage from cortexone increases greatly with age[21] and the increase in sensitivity, associated with dietary overloading and with partial nephrectomy, is well known. Finally, the senile breakdown of the kidney of the rat is accompanied by a marked loss of muscle potassium.[22] Obese rats with disease of the kidney show the same loss of potassium from their muscles.

Essentially the same is true of the parathyroid. The gland is hypertrophied. The kidneys of old rats are more sensitive to damage from hypercalcemia or from phosphate overloading than those of younger animals, and the renal damage which results is similar to that induced by cortexone or chronic potassium deficiency.[23] The skeleton of senile and obese animals becomes demineralized, and Morrison et al.[24] reported that extensive partial nephrectomy even in young rats eventually leads to a condition resembling renal rickets.

It seems probable, therefore, that both the adrenal cortex and the parathyroid become overactive in chronic kidney disease of the type which has been considered, and that their overactivity contributes to the more rapid destruction of any healthy kidney tissue that remains. These endocrine cycles, however, are concerned only in the terminal stages of a destructive process which seems to be the inevitable long term consequence of the initially useful adaptive renal hypertrophy caused by overfeeding.

SUMMARY

Apart from adiposity, overnutrition causes metabolic and structural adaptations in a variety of tissues. Although initially beneficial, such adaptation may finally predispose to pathologic changes. It is suggested that prolonged overproduction of insulin may be a factor in the increased incidence of diabetes in obesity. Possible causes for abnormal tubular hyperplasia observed in the kidneys of obese rats are considered in relation to the frequency of renal diseases in the obese animals.

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DISCUSSION

Dr. Vincent P. Dole (*New York, New York*): Is it possible to fractionate the stress of overfeeding?

It is interesting to ask whether the lesions have any similarity to the lesions seen in animals fed diets excessively high in protein. You are, perhaps, familiar with the old work of Newburg, Sherman and others, who induced a renal lesion from high-protein feeding while seeking an optimum diet.

When these animals are made hyperphagic, there is more than one variable, because they could be permitted to eat a very large number of calories of a mixture very low in protein and thereby obtain an excessive amount of calories as compared to normal and yet still have a normal intake of protein. We do know, of course, that the level of protein feeding affects the metabolic activity in the kidney, will produce hypertrophy, increase the blood flow, and so on.

Have you or do you plan to fractionate the diet by trying different dietary patterns?

Dr. Kennedy: The stock diet that I am using contains only some 13 per cent of protein, anyway, which is just about as little as the rat will exist on. If one increases the amount of protein, lesions do develop much more rapidly in the obese rats. In other words, if one gives a diet with a protein content of 20 per cent, or 25 per cent, things occur much earlier.

The same thing, of course, is true if one increases the sodium chloride content. Dr. Ingle has demonstrated that there is a relationship between salt overloading and the development of lesions.

I am sure that end products of protein catabolism are among the main things excreted, and that the principal source of, if you like to use the old term, renal osmotic work is one of the major factors. I do not think it is, by any means, the only loading factor. But, nevertheless, I would agree that this is the same sort of lesion that Dr. Addis and various other people have studied, and it is essentially an overloading process.

Dr. Dwight J. Ingle (*Chicago, Illinois*): For a number of years now, we have been interested in the possible role of the adrenal gland in the etiology of the so-called adaptation diseases. You are probably all familiar with the kinds of evidence which support the concept that the adrenal is the villain in the etiology of these diseases. I am going to review, very briefly, some of the reasons for doubting this theory.

If the adrenal were the source of the trouble, these diseases should be produced at will by subjecting animals to naturally occurring stresses, but this is not so. The only stress which is effective is exposure to cold, and in such an environment these animals double their intake of food. If you limit the intake of salt of these animals, you may maintain their caloric intake at a high level, and very little more damage occurs than to the control animals. If you overfeed the animals at room temperature, there is almost as much damage in the heart and kidneys and almost as much hypertension as occurs in animals that are exposed to cold.

Also, if this concept were correct, one ought to be able to reproduce it by administering ACTH. Waxler and Miller, in fact, have claimed that administering small amounts of ACTH will induce a remarkable amount of damage, but they neglected to indicate, in their studies, that the same amount of damage occurs in animals that do not receive ACTH. These animals were aging female rats which were obtained from Sprague-Dawley. They have modified their reports a little recently, stating that these animals must be unilaterally nephrectomized. Unilateral nephrectomy does make, as Dr. Kennedy pointed out, changes in the remaining kidney more likely to occur. Such changes will occur within a shorter period of time. We do not disagree with that point. But we have administered a wide range of dosage of ACTH without noting any increase in the incidence of damage.

Finally, if the adrenal were as important as Selye states, we should not be able to produce these diseases in the absence of the adrenal, but we can in the salt-loaded animal. We have papers (in press) indicating that if salt-loading is continued long enough, hypertensive vascular disease will develop in an adrenalectomized animal without substitution therapy.

The adrenal gland is important, however. Adrenalectomy does protect the animal, to a significant extent, against the development of these diseases. We think this is so because the adrenal-insufficient animal now rids itself of a salt load more readily than does an animal having even maintenance doses of adrenocortical extract.

We have tried repeatedly to increase the incidence and severity of these diseases by subjecting animals to various kinds of stress. Our experiments are of short term, never extending beyond two months. They should be continued for a year or more. But it is easy to produce great damage by administering an overdose of DCA for a month.

Let me add one more point. About a year ago, my son, David Ingle discovered, in respect to steroid diabetes produced by an excess of cortisone, that if the animal became host to a tumor, the glycosuria became very mild, and if the tumor grew to a large size, the glycosuria would disappear. As a matter of fact, if the tumor was allowed to grow (this was a Walker

carcinoma) for as long as twelve days and before administering cortisone, no dose of cortisone up to 45 mg. a day would induce glycosuria in these animals.

More recently we have attempted to discover whether stress might suppress other symptoms of hypercorticalism. Stresses do, indeed, suppress them; that is, there are symptoms of hypercorticalism that can be suppressed rather than aggravated if you simultaneously subject the animal to stress, rather than to aggravate it. This is a complex reaction. There are other responses that are aggravated.

DR. KENNEDY: I believe that stress has nothing do with necessarly to adrenal overactivity.

DR. THEODORE VAN ITALLIE (*New York, New York*): I would like to ask Dr. Kennedy whether he made any measurements of blood pressure in these rats, and whether he did any studies of renal function, such as PSP excretion or whatever else can be done in the rat.

It is interesting that obese human subjects do have a higher incidence of hypertension and, more important, obesity seems to potentiate hypertension in man. Is there any parallel in the rat?

DR. KENNEDY: Yes. We wasted a great deal of time trying to get reliable blood pressure determinations by the tail cuff method, the plethysmograph method, in these animals. The tail gets thickened and ulcerated, and we were not successful. I have been obtaining terminal determinations of blood pressure from the aorta. The hypertension seems to be very irregular. Many of these animals are normotensive. There is nothing very striking about the vascular component of the renal lesions.

As for renal function tests, Dr. Stevenson can tell you all about those because he did extensive renal function tests on these animals and reported them a few years ago at the Laurentian Hormone Congress. We did not believe we could improve on the studies he had done. As far as I remember, the main result was a striking decline in glomerular filtration rate as could be expected in a glomerular lesion. I have forgotten what clearance he did use. There was nothing particular that one could learn about the type of disorder of renal function.

DR. J. A. F. STEVENSON (*Toronto, Ontario, Canada*): We discovered that the glomerular filtration rate, as measured by the creatinine clearance test in the rat, decreased in the hypothalamic-hyperphagic animal, as did the renal plasma flow, as measured by the PAH clearance test. This was a gradual decrease over the period of a year to eighteen months that these animals were studied. However, we noticed a marked upset in the water balance of these animals.

I noticed that Dr. Kennedy did not mention the posterior pituitary antidiuretic hormone in any way, and that he did not mention whether or not hypothalamic-hyperphagic animals who are prevented from becoming obese by restriction of food intake exhibit an increased incidence of these renal lesions as compared to the control animals.

DR. KENNEDY: I have not observed any diminution in water exchange in my animals. I think this is a peculiarity from laboratory to laboratory. I believe that lesions in the right section of the hypothalamus do produce hypodipsia, but I was not aware that Dr. Stevenson attributed this to a renal disorder. I think he felt this was an early change of hypothalamic origin.

As to the second point, I do not know what the answer is. I have tried keeping these animals on paired feeding, but to get significant numbers fed daily and restricted for a period of six to nine months is difficult. One would need fairly large numbers to make any useful comparison between the really obese animal and the hypothalamic animal which is non-obese. This is, by no means, a 100 per cent lesion.

DR. JAMES M. SALTER (*Toronto, Ontario, Canada*): We had some observations on this problem, although we were not directly interested in the effect of obesity on the animal. We had rats that were moderately obese through the effects of insulin. They weighed only about 800 or 900 gm. They had a great deal of renal disease and cardiovascular disease, on a normal chow diet. And yet we had animals on a high-fat-diet weighing between 1,400 and 1,700 gm. (one weighed 2 kilos) that outlived the normal control rats. The sections indicated nothing at all. These rats were killed eventually, and pathologic findings were not remarkable. These rats weighed up to 2 kilos and had been that way for up to two years. They were three years old at the time of the study.

DR. STEVENSON: I think that this hypodipsia and lack of water turnover has been observed by most people who have measured it in these animals. It gives rise to a very scanty flow of urine. I might say that in all the renal clearance tests performed on these animals, there was marked evidence of concentration of urine in the renal tubule. The animal seems to be excreting as highly concentrated urine as possible twenty-four hours a day for a year or so. We wondered whether or not this might contribute to the increased pathologic findings in the kidneys.

In our experience, animals with lesions in this region of the hypothalamus, whether or not they become obese, reveal an increased incidence of renal damage, and animals which would become obese but are kept from it by restricted feeding also tend to have this increased incidence.

We believe that it might be this tremendous concentration of the urine throughout the rest of the animal's life which would contribute to the increased incidence of renal damage rather than obesity.

DR. KENNEDY: We often have animals with diabetes insipidus, and I have been interested in producing these. The obese animal which also has diabetes insipidus and is producing a dilute urine has kidney lesions, in my experience, just as frequently as the rat which has a normal water exchange.

DR. MAYER: I would like to underline the variabil-

ity of association of hyperphagia and hypodipsia. We have just sorted out a fairly large group of hyperphagic animals. In a large group of hyperphagic animals we found a certain fraction of them which were extremely hypodipsic, drinking less, in absolute amounts, than normal animals; others which were relatively hypodipsic in terms of the ratio between eating and drinking; and still another group which were not particularly hypodipsic, or, perhaps, hyperphagic and hyperdipsic at the same time. I think that this may account, in part, for variability. We have not as yet correlated these variations with anatomic localization, but we hope eventually to do so.

In terms of the stress of overeating versus the stress of being obese, in obese hyperglycemic mice we have observed that while they live a shorter time than the normal animals, their life expectancy can be considerably reduced if they are subjected to alternate feeding and fasting, and feeding and under-feeding again, so that the weight goes up and down. This may have some practical significance, because so many patients really are on this up and down curve. Perhaps they would do better if they stayed obese if they are not going to be able to prevent themselves from becoming obese again.

DR. F. X. HAUSBERGER (*Philadelphia, Pennsylvania*): I would like to mention that we found similar lesions in older rats with alloxan-diabetes, after diabetes of about six months' duration. Of course, these rats eat much more than normal rats and drink more. That brings in the influence of adrenals again.

By administering cortisone, 2 mg. per day, the incidence of these kidney diseases may be aggravated. Again, these animals, due to the treatment with cortisone, excrete more sugar and lose weight, and they try to compensate by eating more and drinking more.

Reviews of Recent Books

Food for Better Performance, by R. C. Hutchinson. Cambridge University Press, New York, 1958, pp. 102, $2.75.

All of us want to eat well and live well, of course. There are many problems in selecting scientific criteria of "eubiosis," usually equated with freedom from disease and with longevity, and in documenting their relations to nutrition. But living well also involves well-being and good performance. While this constitutes the heart of the popular interest in human nutrition, the nutritional scientist is likely to be ill at ease when these subjects are brought up. The problem of the criteria of "goodness" raises perplexing questions, and rigorous experimentation in this area is difficult at best, if not impossible.

Dr. Hutchinson's aim is not to tackle the tricky problems of methodology or to add to the fund of existing factual information. He is writing for the intelligent layman rather than for his professional colleagues. Consequently, he introduces his presentation by chapters on the nutritive function of the more important food components, and on food utilization and energy requirements. A discussion of alterations of body weight is also inserted, without taking sides on the potential conflict between the definition of the desirable weight as "the weight at which one looks, feels and performs best" and, again, as an actuarial norm.

The core of the volume is the last three chapters dealing with physical activity and foods, mental activity and concentration and the desirable feeding patterns, respectively. The author's principal counsel will not arouse a stormy controversy, as far as its wisdom is concerned: Avoid the extremes. Adverse effects of an empty stomach or of overeating on performance can be combated by reducing the quantity of food eaten at each meal and increasing the frequency of meals to five or six by the addition of mid-morning and mid-afternoon snacks and light late supper. He warns the reader that the food eaten at the main meals must be adjusted downward so that the total amount of food consumed is not increased, advice easier to give than to follow.

Nutritionists will most likely believe that the evidence concerning the virtues of the proposed eating pattern, especially of what the author delightfully calls the "tea breaks," is less solid than one would like. In particular, the effects of rest-pauses alone, of fluid intake that has pharmacologic action but no nutritional value, and of food intake of specified caloric value and nutrient composition, are not clearly separated.

Those concerned with the effects of food on industrial performance will be uncomfortable when looking at a graph (p. 77) which is supposed to represent "a typical daily production curve for a routine industrial operation in which the human element plays a prominent role." The ordinate is not quantified. If, as the author states, "the data from which the curve was drawn were such that a quantitative comparison could not be made," why then proceed to make just such a comparison between the working rates in the early afternoon following a light snack (a good thing) and a full meal (a bad idea)?

Frank G. Boudreau stated fifteen years ago that "there was and still is sore need for controlled studies to throw light on the influence of diets and of various food factors on health and work output." The statement is not much less valid today, especially as far as performance is concerned. For the lay reader Dr. Hutchinson has summarized much that is known. Perhaps the chief value of his book for the professional reader will be the questions that remain in regard to industrial nutrition in general and in regard to fatigue (with depressed production rates toward the end of the morning and afternoon work period) and boredom (with decreased output at the middle of each work period), in particular. If mid-period snacks alleviate both fatigue and boredom, the mechanisms are probably different. In boredom the role of food is likely to be personal (emotional) and interpersonal rather than biochemical. This would not make, of course, the between-meal feeding any less practically relevant.

J. Brožek

The Megaloblastic Anemias, by Victor Herbert. Grune & Stratton, New York, 1959, pp. 181, $6.00.

In this relatively short monograph the author has prepared a thorough discussion of megaloblastic anemias. Based on over 600 references, many exceptionally current, the review classifies megaloblastic anemias due to vitamin B_{12} deficiency under the main headings of inadequate ingestion, defective absorption and inadequate utilization. A similar etiologic classification is used for folic acid deficiencies. The clinical picture, differential diagnosis and therapy are also fully presented.

Most of the concepts are orthodox according to current views. However, Dr. Herbert believes the term "megaloblastic anemia of pregnancy" should be discarded for reasons clearly stated. He also suggests that approximately 6 μg. a day is a better estimate of the maintenance requirement of vitamin B_{12}. Large

parenteral doses of the vitamin are recommended in the initial treatment of patients with this deficiency.

The author discusses only briefly his own views on the role of intrinsic factor in vitamin B_{12} transport (*Am. J. Clin. Nutrition*, 7: 433, 1959) and indeed it can be said that the many conflicting observations and opinions in this field are all fairly represented.

This is a neat, thoughtful and complete treatise on a clinically important topic and the book can be recommended both as a reference source for investigators and as a useful practical guide for clinicians. S. O. W.

Medical Discoveries: Who and When, by J. E. Schmidt. Charles C Thomas, Springfield, Ill., 1959, pp. 555, $14.75.

The author, according to the jacket of his new book, is President of the American Society of Grammatolators. (A grammatolator is an idolator of words.) Dr. Schmidt's book is a dictionary listing about 6,000 medical and related scientific discoveries. Each entry gives the name of the discoverer, his profession, nationality, floruit (i.e., 1777–1858) and the date of the discovery. By perusing this text, one can learn, for example, when ipecac was introduced in Europe, who first recognized ozena as a clinical entity, or who introduced the wool skein test for color vision. It is evident that much research has gone into preparing the entries. The author prides himself on having unearthed the given name as well as the surname of most of the discoverers.

In a treatise of this magnitude, one can find several items to quibble about. "Vitamin B_3, discovery of" and "Vitamin B_5, discovery of," for instance, will send most of us to other sources to find the meaning of these obsolete terms. The insertion of cross indexed references to two other dictionaries (recently published by Dr. Schmidt) in a compendium of medical discoveries would seem somewhat questionable, as would the entry "Cesium, effect of, on rate of oxidation of sewage" to describe an observation by the author himself.

On the whole, this book offers an unusual source of valuable information about many important and relatively obscure discoveries. It should prove useful as a reference text. J. E. WING

Vitamins and Hormones. Advances in Research and Applications, Vol. XVII, edited by Robert S. Harris, G. F. Marrian and Kenneth V. Thimann. Academic Press Inc., New York, 1959, pp. 324, $14.00.

The seventeenth volume of this well known series of essays reflects the thin boundaries between nutrition, endocrinology and metabolism. Ashmore and Weber, for example, discuss the role of the enzyme, glucose-6-phosphatase in carbohydrate metabolism. This substance is affected by various hormones. Estrogens, serotonin and ergothioneine are also discussed.

Dalderup points out certain interesting similarities between atherosclerosis and toxemia of pregnancy. The biochemistry of the vitamin K group and the metabolism of folic acid round out the volume.

The usual high standards of this series have been maintained and again readers will find this volume a useful and authoritative collection of reviews.

S. O. W.

History of the American Dietetic Association, by Mary I. Barber. J. B. Lippincott Co., Philadelphia, 1959, pp. 328, $6.00.

This book is the official history of the American Dietetic Association since its organization in 1917 to the present. It relates the growth in membership from the ninety-eight persons attending the first Dietitian's Conference in Cleveland to approximately 14,000 members in 1959. The author is well qualified to write such a history, for she has been a most active member in many capacities. She has drawn freely from articles in the Association's journal and from the detailed records of Mrs. Anna Boller Beach.

A publication such as this undoubtedly serves the membership of the Association. Many will experience a sense of nostalgia on reading it; they may justifiably take pride in the accomplishments in less than half a century. This reviewer, however, believes that the book serves other useful purposes. It serves as an objective record of the growth of a profession. The Association has fostered higher standards of academic preparation for the dietetic profession; has established a nationwide internship program; has set high levels of performance in the areas of administration, community nutrition, diet therapy and education so that food service for the well and the sick continues to improve; has established criteria for nutrition and dietetic courses in nursing and medical curricula; and has published for thirty-five years a journal which is authoritative in content and held in esteem by dietitians and nutritionists in medical circles and by food service groups. As an inspiring record of accomplishment, the book may well serve to enlighten young women seeking a career in a profession of ever-increasing prestige, where the demands far exceed the supply. This book will serve as a valuable reference in public and school libraries.

C. ROBINSON

Gouty Arthritis and Gout. An Ancient Disease with Modern Interest, by Thomas E. Weiss and Albert Segaloff. Charles C Thomas, Springfield, Ill., 1959, pp. 221, $7.50.

"This compilation of theories, facts, clinical observations, pathology, treatment, and extensive bibliography, was undertaken to furnish the student, clinician, and investigator with a usable reference on gout." This quotation from the preface of the book adequately summarizes the contents. The authors have succeeded in carrying out their intentions as outlined in the preface.

The book is divided into well organized chapters so that one may use it with ease and efficiency as a source of reference for the many aspects of gout and gouty arthritis.

The largest portion of the book is concerned with a

description of the meticulous treatment of all phases of gouty arthritis. For this reason, the book will probably be of most value to clinicians. It is highly recommended to all physicians interested in this intriguing disease. K. R. CRISPELL

Evaluation of Frotein Nutrition. Report of the Food and Nutrition Board. Publication 711 of the National Academy of Sciences—National Research Council, Washington, D. C., 1959, pp. 60, $2.00.

This important monograph was prepared by the Committee on Amino Acids of the National Research Council Food and Nutrition Board. The members are Drs. Andrews, Follis, Jr., Harper, Hegsted, Holt, Jr., Phipard, Williams and Allison (chairman). It is an evaluation of dietary protein meals which surveys protein and amino acid requirements, effects of deficiencies and (interestingly) excesses, dietary content and amino acid supplementation.

Of particular current interest is the discussion on lysine supplementation. In general the Committee does not anticipate any improvement of the normal diet in this country by supplementation of cereals with lysine.

Much attention is paid to the concept of biological value, the percentage of absorbed nitrogen retained in the body. On this basis the relative "values" of proteins are compared.

This is a clear and concise summary of the protein problem in the United States today. It may be taken as the considered opinion of some of the best informed workers in the field. S. O. W.

Nutritional Diagnosis, by Grace A. Goldsmith. Charles C Thomas, Springfield, Ill., 1959, pp. 164, $5.50.

This concise, well written monograph provides an up-to-date and authoritative account of nutritional diagnosis. Clinical recognition of nutritional disease is emphasized with attention to pertinent biochemical alterations, pathophysiology and therapy. An introductory chapter provides the reader with the background and philosophy of the author who is an expert in this field. There are well documented discussions on caloric undernutrition and obesity; protein, carbohydrate and lipid metabolism; and mineral and vitamin nutrition. A short chapter on the future of nutritional diagnosis stresses the importance of being cognizant of both primary nutritional diseases and of nutritional observations in other diseases. The text is clearly and interestingly written. Both the index and a selected bibliography are adequate.

This monograph is a welcomed addition to medical literature. Its brevity and simplicity will make it a valuable book for medical students, practitioners and members of allied professions who are interested in nutrition. C. M. LEEVY

BOOKS RECEIVED

Books received for review by *The American Journal of Clinical Nutrition* are acknowledged here. As far as practicable, those of special interest are selected, as space permits, for review.

World Review of Nutrition and Dietetics, edited by Geoffrey H. Bourne. J. B. Lippincott Co., Philadelphia, 1960, pp. 272, $12.00.

Ciba Foundation Study Group No. 4. Virus Virulence and Pathogenicity, edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Little, Brown & Co., Boston, 1960, pp. 114, $2.50.

Kochsalzarme Kost, by H.-J. Holtmeier. Georg Thieme Verlag, Stuttgart, 1960, pp. 416, DM 39 ($9.30).

From Fish to Philosopher. The Story of Our Internal Environment, by Homer W. Smith. Ciba Pharmaceutical Products, Inc., Summit, New Jersey, pp. 304.

Food Enrichment in South Africa. South African Council for Scientific and Industrial Research, Pretoria, South Africa, 1959, pp. 157, 21*s*.

Basic Facts of Body Water and Ions, by Stewart M. Brooks. Springer Publishing Co., New York, 1960, pp. 159, $2.75.

Food Preferences of Men in the U. S. Armed Forces, by David R. Peryam, Bernice W. Polemis, Joseph M. Kamen, Jan Eindhoven and Francis J. Pilgrim. Department of the Army, Chicago, 1960, pp. 160.

Vitamin B_{12}, by Lester E. Smith. John Wiley & Sons, Inc., New York, 1960, pp. 196, $3.00.

Anorexia Nervosa. Its History, Psychology, and Biology, by Eugene L. Bliss and C. H. Hardin Branch. Paul B. Hoeber, Inc., New York, 1960, pp. 210, $5.50.

Annual Review of Medicine, Volume 11, edited by David A. Rytand. Annual Reviews, Inc., Palo Alto, Calif., 1960, pp. 453, $7.00.

Essentials of Fluid Balance, 2nd edition, by D. A. K. Black. Charles C Thomas, Springfield, Ill., 1960, pp 135, $4.50.

British Medical Bulletin, Volume 16, No. 2. The Thyroid Gland. British Council, London, May 1960, $3.25.

The Thyroid-Vitamin Approach to Cholesterol Atheromatosis and Chronic Disease: A Ten-Year Study, by Israel Murray. Vascular Research Foundation, New York, 1960, pp. 132.

Nutritional Evaluation of Food Processing, edited by Robert S. Harris and Harry von Loesecke. John Wiley & Sons, Inc., New York, 1960, pp. 612, $12.00.

Aids to Biochemistry, fifth edition, by S. P. Datta and J. H. Ottaway. Ballière, Tindall & Cox, London, 1960, pp. 266, $3.75.

The Chemical Senses in Health and Disease, by H. Kalmus and S. J. Hubbard. Charles C Thomas, Springfield, Ill., 1960, pp. 95, $3.75.

The Year Book of Endocrinology, edited by Gilbert S. Gordan. Year Book Publishers, Inc., Chicago, 1960, pp. 384, $8.00.

Medicinal Chemistry, 2nd edition, edited by Alfred Burger. Interscience Publishers, Inc., New York, 1960, pp. 1243, $37.50.

Abstracts of Current Literature

CALCIUM METABOLISM

Calcium excretion in stool and urine does not have any specific regulatory control. In feces, the calcium is derived both from diet and from intestinal secretions. In the urine, calcium levels are controlled by endogenous factors characteristic of the individual. Increased calciuria following the administration of hydrocortisone has been observed and may be useful in modifying serum levels in hypercalcemic states.

Studies in Calcium Metabolism. I. Clark and R. Geoffroy. *J. Biol. Chem.*, 233: 203, 1958.

Calcium excretion was studied in rats treated with single doses of hydrocortisone, parathyroid extract, vitamin D, $MgSO_4$, Na_2SO_4, NH_4Cl and sodium citrate. $MgSO_4$ or sodium citrate given subcutaneously two days after an injection of Ca^{45} caused an immediate rapid excretion of the radioactive calcium. Parathyroid extract did not exert its effect until eight hours after administration. Administration of hydrocortisone resulted in hypercalciuria for the first four hours, followed by a low excretion rate up to forty-eight hours. Moderate hypercalciuria resulted from the administration of Na_2SO_4, and only slight increases in Ca^{45} excretion were observed with the administration of NaCl or NH_4Cl. Injections of parathyroid extract, $MgSO_4$ or sodium nitrate, when given sixty days after the administration of Ca^{45}, caused a six- to tenfold increase in urinary radiocalcium and a two- to threefold increase in excreted Ca^{40}, measured over a period of eight days. These substances appeared to have a specific osteolytic action on the bone since they were effective either two days or sixty days after the administration of Ca^{45}, and since any increase in Ca^{45} excretion after sixty days must come from bone. Hydrocortisone slightly depressed calcium excretion for four days before an increased excretion of both Ca^{45} and Ca^{40} appeared. This, plus its activity in short term experiments, suggested that hydrocortisone tends to suppress the deposition of calcium into bone without affecting the normal rate of resorption.

M. K. HORWITT

The lack of effect of certain hormones upon calcium balance may be attributed to an inadequate period of observation. It is necessary to extend such studies over long intervals to demonstrate any effect.

Effect of Some Hormones on Calcium Balance in Elderly Subjects. G. Toro, P. G. Ackermann and W. B. Kountz. *Proc. Soc. Exper. Biol. & Med.*, 97: 819, 1958.

Thyroid 30 mg. daily, protamine zinc insulin 10 units daily, cortisone 100 mg. daily and corticotrophin 30 mg. daily were given one at a time to five subjects aged between sixty-nine and eighty-eight years who were free of acute and infectious diseases. None of these hormones had an appreciable effect on calcium balance.

G. WALKER

The use of strontium in the treatment of osteoporosis has not met with favor. The metabolism of calcium and strontium appears to be similar. However, the basic problem in this disease may not be one of calcium deficiency.

Interrelationship Between Serum Calcium Levels: Calcium^{45} and Strontium^{85} Metabolism in Man. G. Mazzuoli, J. Samachson and D. Laszlo. *J. Lab. & Clin. Med.*, 52: 522, 1958.

These interesting studies were undertaken to determine the effect of serum calcium levels on the absorption and excretion of calcium and strontium. Two patients in a normocalcemic state were given a mixture of calcium^{45} and strontium^{85} orally. Plasma levels as well as fecal and urinary excretion of these isotopes were

measured over a period of days. Similar measurements were made with the same patients in a hypercalcemic state produced by the infusion of calcium gluconate and in a hypocalcemic state produced by an infusion of sodium ethylenediaminetetraacetate.

Acute changes in the serum calcium levels had no significant effect on the absorption of either isotope. In all the studies more calcium^{45} than strontium^{85} was absorbed and the fecal excretion of calcium^{45} was slightly lower. In the control studies, the urinary excretion of strontium^{85} was considerably greater than that of calcium^{45}. During hypercalcemia the urinary excretion of both tracers was increased; in the hypocalcemic period, a marked increase in calcium^{45} excretion accompanied a marked decrease in strontium^{85} excretion. G. HOLLIFIELD

The physicochemical state of serum calcium is of great importance in determining whether or not hypocalcemic symptoms occur in the presence of low serum levels. In renal disease, abnormal reductions in serum calcium do not often result in symptoms because of a relatively high level of ultrafiltrable calcium usually ascribed to the lowering of the blood pH.

The Ultrafiltrable Calcium of Human Serum. II. Variations in Disease States and Under Experimental Conditions. A. R. Terepka, T. Y. Toribara and P. A. Dewey. *J. Clin. Invest.*, 37: 87, 1958.

Serum calcium is composed of two major fractions: the protein-bound calcium which is non-diffusible and the ultrafiltrable calcium which is diffusible. The latter contains predominantly ionic calcium and participates in metabolic processes at the cellular level. In this study the ultrafiltrability of serum calcium was determined in disease conditions associated with high and low serum calcium levels as well as before, during and after the parenteral administration of calcium, citrate, phosphate and parathyroid extract.

It was found that an increase in serum calcium, whether due to a disease or secondary to administration of either calcium or parathyroid extract, caused a proportionate increase in both ultrafiltrable and protein-bound calcium. A state of hypocalcemia was accompanied usually by a high percentage of ultrafiltrable calcium. This relationship did not always exist in renal disease. A decrease in protein-bound calcium may occur because of the fall in serum protein concentration, or a specific alteration in the binding ability of proteins, or a decrease in the ionized calcium level.

In renal disease a high percentage of ultrafiltrable calcium was always found, regardless of the level of serum proteins.

Abnormalities in the concentration of ultrafiltrable calcium in serum are ascribed to disturbances of the equilibrium of bone-to-extracellular fluid calcium. Abnormalities in the percentage of ultrafiltrable calcium (the ratio of ultrafiltrable to total serum calcium) are related to alterations in calcium-protein interrelationships. S. O. WAIFE

Calcium and Phosphorus Balances in African Children. K. Holemans and A. Lambrechts. *J. Trop. Pediat.*, 4: 43, 1958.

The authors have studied calcium and phosphorus balance in African children living on low intakes of these minerals. The results have shown that the maintenance requirements for these two substances are not lower than those of white children, and are 12 mg./kg./twenty-four hours for calcium and 21 mg./kg./twenty-four hours for phosphorus. On intakes above the minimum, fairly high retentions of calcium and phosphorus have been observed. It is suggested that the observed high retentions are the expression of mineral depletion of the children studied, despite the absence of clinical evidence of depletion. J. C. WATERLOW

The Mineral Content of Normal Human Bone. J. W. Agna, H. C. Knowles, Jr. and G. Alverson. *J. Clin. Invest.*, 37: 1357, 1958.

Although the human skeleton plays a role in electrolyte metabolism, information regarding human bone is sparse. This study was concerned with the composition of bone obtained at autopsy from sixteen normal adults who died suddenly. Analysis of skull, rib and ilium showed that the skull contained significantly greater amounts of calcium, phosphorus, carbonate and sodium but lesser amounts of water, nitrogen, chloride and potassium than did the ilium. The composition of rib was intermediate between skull and ilium. S. O. WAIFE

METABOLIC ROLE OF VITAMIN B_{12}

It is now believed that intrinsic factor is required not only for the absorption of vitamin B_{12} but also for its retention by tissues. Substances with intrinsic factor activity enhance the vitamin-binding capacity of serum (α_2- and β-globulins) and increase the level of tissue uptake of the vitamin. In the gastrointestinal tract it has been shown that the "acceptor factor" for transport of vitamin B_{12} from lumen to plasma may be concentrated in the ileal region.

The Site of Absorption of Vitamin B_{12} in Man. C. C. Booth and D. L. Mollin. *Lancet*, 1: 18, 1959.

The site of vitamin B_{12} absorption is not known. It has been suggested that it is in the proximal part of the small intestine but the clinical observation that absorption is hindered when the distal part is diseased contradicts this.

In the study reported here the distribution of vitamin B_{12} labeled with radioactive Co was traced at laparotomy three hours after oral administration to four subjects. Vitamin B_{12} absorption was studied in an additional twelve subjects who had undergone resection or short circuit of the small bowel.

In the first four subjects all the vitamin B_{12} had passed to the distal part of the small intestine providing strong evidence that the ilium is the site of absorption since none is absorbed in the large bowel. The study of the

other twelve subjects confirmed this view. When extensive surgical treatment affecting the lower end of the small intestine has been carried out, vitamin B_{12} absorption is invariably subnormal and is unaffected by either intrinsic factor or previous treatment with chlortetracycline. In many of these patients there was evidence of vitamin B_{12} deficiency. The authors draw attention to the fact that patients who undergo surgery of the ilium should either be kept under close hematological supervision or treated prophylactically with vitamin B_{12}. F. E. HYTTEN

Influence of Calcium Ions on Vitamin B_{12} Absorption in Steatorrhea and Pernicious Anaemia. R. Gräsbeck, I. Kantero and M. Siurala. *Lancet*, 1: 234, 1959.

It has been shown that calcium ions are probably necessary for the absorption of vitamin B_{12} and the suggestion has been made that impaired absorption in steatorrhea is due to removal of calcium from the gut as insoluble soaps. In six subjects (aged one to sixty-two years) with steatorrhea, and six subjects with pernicious anemia (aged fifty-nine to seventy-two years), absorption of Co^{60}-labeled vitamin B_{12} was measured with and without an accompanying dose of calcium lactate. The calcium considerably improved absorption of vitamin B_{12} in the patients with steatorrhea but had no obvious effect in those with pernicious anemia. F. E. HYTTEN

Intrinsic factor inhibiting substances have been demonstrated in serums of patients with pernicious anemia who were treated with vitamin B_{12} and pig pylorus mucosa (Schwartz: Lancet, 2: 61, 1958). It is possible that inhibition is not due to antibody formation but is produced by an extraneous protein with binding capacity.

Observations on the Inhibitory Effects of Intrinsic Factor Preparations on Vitamin B_{12} Absorption. K. B. Taylor, B. J. Mallett and G. H. Spray. *Clin. Sc.*, 17: 647, 1958.

It has been reported that some preparations of pig intrinsic factor may inhibit the absorption of vitamin B_{12} in human subjects, but the mechanism is not understood. Large amounts, from 400 to 1,400 mg. of an impure preparation of pig intrinsic factor, inhibited the absorption of vitamin B_{12} in both normal subjects and patients with pernicious anemia. The inhibition was not related to the size of the dose of the vitamin.

Inhibition persisted with intrinsic factor in which intrinsic factor activity had been destroyed by heat; the capacity to bind vitamin B_{12} was not affected. "It appears that the inhibition may be due either to oversaturation of some intestinal acceptor mechanism or to the presence of vitamin B_{12}-binding material contaminating the intrinsic factor, or to both."

F. E. HYTTEN

Great interest has focused on the observation that D-sorbitol enhances the absorption of vitamin B_{12} in normal males and in animals. The mode of action has been suggested as increasing intrinsic factor action of gastric secretions. A similar effect should be sought to explain the results obtained with the administration of gentian violet.

Absorption of Vitamin B_{12} in a Patient with Intestinal Stasis: Improvement by Gentian Violet and Antibiotics. R. L. Wolf, E. A. Brody and S. Estren. *New York J. Med.*, 59: 1531, 1959.

A twenty-three year old Puerto Rican woman had a blind loop of ileum as a result of a postappendectomy adhesion. There was a marked anemia predominantly due to iron deficiency. There also was low serum vitamin B_{12} levels, rare macrocytes and large hypersegmented polymorphonuclear leukocytes in the peripheral blood. There was practically no absorption of vitamin B_{12} by the Schilling technic. Intrinsic factor material prepared from desiccated hog stomach was added; there was no change. However, with the addition of neomycin and Aureomycin® there was improvement in vitamin B_{12} absorption. Finally, the administration of gentian violet led to absorption of 2.4 per cent of the ingested dose of radioactive vitamin B_{12} in twenty-four hours. According to the authors, these data support the hypothesis that intrinsic factor and Aureomycin were not useful in this case but that neomycin and gentian violet, by increasing the absorption of vitamin B_{12}, support the hypothesis that antibiotic or antibacterial agents act by decreasing bacterial competition for vitamin B_{12} in patients with intestinal stasis. S. O. WAIFE

The practice of administering vitamin B_{12} in the treatment of a wide variety of conditions for which no indications exist is rather commonplace. The fallacy of this trend, except for purposes of placebo therapy, is emphasized in this report.

Serum and Tissue Concentration of Vitamin B_{12} in Certain Pathologic States. J. A. Halsted, J. Carroll and S. Rubert. *New England J. Med.*, 260: 575, 1959.

Data from this study do not support the contention that partial or biochemical deficiency of vitamin B_{12} may occur in various chronic diseases or old age. Clinical material consisted of blood serum and various tissue specimens taken at autopsy. For control subjects, medical students, hospital personnel, hospitalized patients and chronic-care patients at a home for aged were studied. All were taking an adequate diet and had hemoglobin values of 11.0 gm. per 100 ml. or more. For the entire control group of 333 subjects, the mean serum vitamin B_{12} concentration was 470 $\mu\mu$g. per ml. There was no sex difference. The data indicate that serum concentration in various chronic diseases is not significantly less than that in control subjects. It is postulated that the so-called favorable effect of vitamin B_{12} therapy in conditions without evidence of deficiency (i.e., without megaloblastic anemia or combined system disease, with serum vitamin B_{12} levels of less than 100 $\mu\mu$g. per ml.) is, "...the result of suggestion, the placebo mechanism." E. COHEN

Serum Vitamin B_{12} Content in Liver Disease. T. D. Stevenson and M. J. Beard. *New England J. Med.*, 260: 206, 1959.

An elevated level of serum vitamin B_{12} is found in patients with acute and chronic liver disease. The increased level may be caused by increased absorption of the vitamin B_{12} from the gastrointestinal tract, decreased urinary excretion of the vitamin, increased binding capacity of the serum for the vitamin, or release of the vitamin from the necrotic liver. In this paper the serum vitamin B_{12} content was determined using a microbiologic assay in twenty-seven patients with Laennec's cirrhosis, seven with hepatitis and five with obstructive jaundice. The serum level of the vitamin was elevated above normal in the patients with cirrhosis and hepatitis but was in the normal range in all patients with obstructive jaundice. Since the absorption, excretion and plasma clearance of the vitamin were normal in the patients with the elevated vitamin B_{12} level, the authors concluded that the increased level is due to a release of the vitamin from the liver as a result of hepatic necrosis or cellular damage. M. W. BATES

Vitamin B_{12} and the Course of Diabetic Retinopathy. H. Keen and R. Smith. *Lancet*, 1: 849, 1959.

Abnormalities of vitamin B_{12} metabolism have been described in diabetic subjects with retinopathy. Although treatment with vitamin B_{12} has been occasionally described, no controlled assessment has been made.

The experiment described here was a carefully controlled study in twenty-five subjects with diabetes, twelve of whom received 150 μg. of vitamin B_{12} daily orally. Some interesting new knowledge of the natural history of the retinal changes is described in detail but no advantage of vitamin treatment could be demonstrated. F. E. HYTTEN

Further studies on oral vitamin B_{12} therapy for pernicious anemia suggest that the method may be less satisfactory than parenteral therapy for maintenance of high serum and normal tissue levels of the vitamin.

Treatment of Pernicious Anemia by Oral Administration of Vitamin B_{12} Without Added Intrinsic Factor. E. A. Brody, S. Estren and L. R. Wasserman. *New England J. Med.*, 260: 361, 1959.

Fourteen patients with pernicious anemia, two with total gastrectomy and one with a malabsorption syndrome were treated orally with large doses of vitamin B_{12} (150 μg. daily) for a period of two to thirty-four months. Eleven patients with pernicious anemia, one with total gastrectomy and the one with the malabsorption syndrome showed complete hematologic and clinical remission. An increase in the serum vitamin B_{12} level, due to an increase in the bound vitamin, was also observed. However, normal levels were reached only in the two patients with gastrectomy and in three patients with pernicious anemia. In the other patients the maximum levels achieved with vitamin B_{12} therapy were low normal or borderline. Although only a small portion of the administered vitamin was absorbed, the needs of the bone marrow and nervous system seemed to be met, but the tissue storage depots were not replenished as reflected by the low serum vitamin B_{12} level. The results obtained were comparable to those obtained with parenteral administration of smaller amounts of vitamin B_{12}. Since the remission occurred more slowly after oral administration of the vitamin, parenteral therapy is preferred when rapid response is essential, when severe neurologic involvement is present and when the patient cannot be trusted to administer his own medication. M. W. BATES

The metabolic reactions known to involve vitamin B_1 include protein synthesis, activation of sulfydryl groups, biosynthesis of methyl groups and deoxyriboside synthesis. Primary and secondary effects of vitamin B_{12} deficiency are, at present, almost impossible to distinguish because of the complexity of the biochemical interrelationships in which it is involved.

Lactic Acid Dehydrogenase in Vitamin B_{12} Deficiency. Genuine Pernicious Anaemia and Pernicious Tapeworm Anaemia. B. Gordin and T. M. Enari. *Acta haemat.*, 21: 16, 1959.

Vitamin B_{12} activity was measured with Lactobacillus leichmannii 313 (ATCC 7830) as well as serum lactic dehydrogenase (LD) in two patients with genuine pernicious anemia and in two patients with pernicious tapeworm (diphyllobothrium) anemia. High values were found during relapse in both. As reticulocyte levels reached peaks LD activity decreased and returned to normal when blood values were still anemic. There was no correlation between hemoglobin or red blood cell values and LD activity. However, there was direct correlation between serum vitamin B_{12} concentration and LD activity. Simultaneously there was an increase in vitamin and reticulocyte values but a decrease in LD. The authors postulate that there is an increased release of LD from the short-lived red blood cells. The correlation between reticulocyte peak and the decrease in LD may substantiate such an explanation. Megaloblasts disappeared within twenty-four to forty-eight hours after initiating adequate therapy; when reticulocytosis attained its maximum, they were no longer found. Simultaneously, the LD was normal.
E. COHEN

Cerebral Manifestations of Vitamin B_{12} Deficiency. J. S. Wiener and J. M. Hope. *J.A.M.A.*, 170: 1038, 1959.

Although it has been known for some time that cerebral manifestations may be present in pernicious anemia, little attention has hitherto been paid to the mental changes occurring in this illness. The lesions apparently consist of a subacute degeneration essentially similar to that occurring in the spinal cord. There have been no reports of cerebral lesions which have *not*

been associated with changes in the spinal cord, and the order of involvement appears to be posterior columns, followed by degeneration of lateral and anterior columns, and finally of the cerebral white matter.

The mental symptoms are not characteristic. They are variable in degree and quality, and range from a mild mood disorder to psychotic behavior.

In this paper the authors describe a forty-four year old woman admitted to the hospital with severe depression, psychomotor retardation and paranoid ideas. Irritability and fatigue were also present. Physical examination revealed pallor, smooth tongue, absent vibratory sense and hyperactive tendon reflexes. Moderate macrocytic anemia was present and the bone marrow was that of normoblastic hyperplasia. No free acid was found in the gastric analysis. The serum vitamin B_{12} was very low, and the Schilling test revealed essentially no excretion of radioactive cobalt. After a few days of intramuscular administration of vitamin B_{12}, there was a marked improvement in her mental behavior. Subsequent follow up revealed a remarkable recovery.

Because the reversibility of the mental aberrations seems to depend on the duration of illness before the institution of therapy, the authors call attention to the need for recognition of this syndrome and for prompt and adequate therapy. S. O. WAIFE

Vitamin B_{12} Therapy of Hemolytic Syndromes. W. Wolf. *Ärztl. Forsch.*, 13: 15, 1959.

Comparative spectrophotometric studies on the osmotic and mechanic resistance of erythrocytes after intramuscular administration of vitamin B_{12} in cases of hemolytic and non-hemolytic diseases gave evidence of the resistance-increasing action (inhibitory effect on hemolysis) of cobalamin. This results in its widened employment as a membrane-active agent in hemolytic syndromes and as a prophylactic agent against hemolysis. The mode of action is discussed and the usual macromethods for the assessment of resistance of erythrocytes are critically evaluated. AUTHOR

Folic Acid Activity in the Liver of Sheep. III. The Effect of Vitamin B_{12} Deficiency on the Concentration of Folic Acid and Citrovorum Factor. M. C. Dawbarn, D. C. Hine and J. Smith. *Australian J. Exper. Biol. & M. Sc.*, 36: 541, 1958.

The interrelationship of folic acid and vitamin B_{12} is a complex subject. In this report analysis was made of the liver of sheep subsiding on a cobalt-deficiency ration (which produced vitamin B_{12} deficiency). These were compared to a control group fed *ad libitum*. In addition, other animals were given the same amount of food (pair feeding) as the deficient animals but were given injections of vitamin B_{12}. It was found that among vitamin B_{12}-deficient animals the concentration of folic acid and citrovorum factor was greatly reduced. The authors conclude that the concentration of folic acid and citrovorum factor in the liver fell precipitately when the vitamin B_{12} concentration dropped below a certain level (0.19 μg./gm.). This was not due to the restricted food intake. S. O. WAIFE

Investigation on the Minimum Requirements of Vitamin B_{12} for Rats During Reproduction. W. G. Jaffé. *Arch. venezol. nutricion*, 8: 119, 1957.

A group of rats has been kept for eighteen generations on a fortified soy bean-corn ration, deficient in vitamin B_{12}. From the second generation on a high mortality rate of the newly born was observed in this group, as well as a low weanling weight, slow growth after weaning and delayed sexual maturity. All these symptoms were overcome when 5 μg. of vitamin B_{12}/kg. of diet were added, while 3 μg./kg. were insufficient to cause normal weaning weight, although sufficient to overcome all the other deficiency symptoms.

During the eighteen generations of observation, no signs of adaptation to the vitamin B_{12}-deficient diet were observed. The reduced glutathione levels in the liver of all the groups of rats studied were normal.

AUTHOR

GROWTH AND DEVELOPMENT

An energy intake, increased above the needs for maintenance and activity, will provide the extra protein, fat and carbohydrate required for growth of body tissues. It is an unusual and highly abnormal diet which will supply the necessary protein and calories yet be inadequate in essential micronutrients such as thiamin and cobalamin. Nevertheless these latter factors have been recommended to increase growth rates of children. The following observations of the Committee on Nutrition are of interest in this connection.

Appraisal of the Use of Vitamins B_1 and B_{12} as Supplements Promoted for the Stimulation of Growth and Appetite in Children. Committee on Nutrition, American Academy of Pediatrics. *Pediatrics*, 21: 860, 1958.

The great demands made on practitioners by parents for the use of "tonic" supplements in children necessitate careful evaluation of claims of efficacy of such substances. Factors affecting appetite are multiple and complex, and involve a particularly wide variety of psychologic and sociologic influences which make evaluation difficult.

Thiamin has not been demonstrated to have a direct effect on appetite, although appetite suffers as a part of the chain of metabolic alterations induced by definite deficiency and is restored when this deficiency is corrected. Such deficiency among American infants and children is exceedingly rare. There is no evidence that thiamin stimulates appetite except in severely deprived subjects. When maternal diet is optimal, breast milk contains adequate amounts of thiamin. Formulas made from commercially processed milk products also contain adequate amounts of this vitamin. In addi-

tion, many foods (e.g., cereals, bread) are fortified with vitamin B_1.

The establishment of a requirement for vitamin B_{12} for normal growth in bacteria, mice, rats, chicks and hogs naturally led to an investigation of the effects of this vitamin in children. Observations have been made on the growth-promoting effects of vitamin B_{12} in premature and term infants, malnourished or chronically ill infants and children and children with "growth failure." The bulk of evidence definitely indicates that vitamin B_{12} has no effect on appetite stimulation or growth promotion. Evidence advanced in support of such specific effects appear to be unacceptable because of lack of scientifically acceptable controlled observations.

Available evidence is insufficient to support the use of either vitamin B_1 or vitamin B_{12} for stimulation of appetite or growth except in deficiency states.

T. C. PANOS

Not only linear growth but also the deposition and distribution of body fat is influenced by hormonal factors.

Fat Changes During Adolescence. S. M. Garn and J. A. Haskell. *Science*, 129: 1615, 1959.

An estimate of the subcutaneous fatty tissue of the lower thoracic area was made in 259 children in Ohio. The data indicate a parallel increase in fat from the sixth through the eleventh year for both boys and girls. Thereafter, fat on the lower thorax continued to increase in girls, reaching the maximum thickness by the fourteenth year, while in boys it was stabilized between the eleventh and seventeenth year. The simplest explanation is that of a steady increase in outer fat in both sexes, with a temporary interruption in the male during the period of steroid hormone differentiation.

S. O. WAIFE

Effects of Orally Administered Stilbestrol upon Growth and upon Calcium and Phosphorus Metabolism in Lambs. J. D. Shroder and S. L. Hansard. *J. Animal Sc.*, 17: 343, 1958.

The favorable effects of stilbestrol upon rate of gain and feed efficiency have been widely accepted, but few attempts have been made to determine the mechanism of action of this material. Twenty-eight wether lambs, averaging 75 pounds, were used in the experiment. To the basal ration, 2 mg. stilbestrol per head was added daily. Calcium^{45} and phosphorus^{32} were given either orally or intravenously. Endogenous calcium and phosphorus losses were measured by the comparative balance procedure of Hansard; true digestibility was calculated by the conventional formula. At the end of the feeding experiments, the animals were weighed and sacrificed, and the right femur bone of each was studied by autoradiograph technics, and by the application of precision calipers.

The gains in weight over the seventy-five–day feeding period were (1) basal ration, 30.4 pounds, and (2) stilbestrol-fed, 34.8 pounds, which was considered by the authors "not significant at the 5 per cent level." However, the authors in their summary conclude that "in the ruminant animal the primary effect of stilbestrol administration was that of growth stimulation."

Bone measurements showed significantly greater growth in length in the stilbestrol-fed lambs only at the distal end. At the proximal end, the differences were not significant. On the basis of centimeters per unit gain in weight, however, there were no differences between the control and treated groups. Expressed as a percentage of body weight, there was no greater amount of total bone in the stilbestrol-fed group.

Calcium retentions in stilbestrol-fed lambs were greater than in the control animals both in oral feeding and when intravenously administered. Phosphorus retention data followed a similar pattern. Apparent digestibility of both calcium and phosphorus was greater in the stilbestrol-fed group, accomplished mainly by lower fecal endogenous losses.

Blood radioactivity studies following the intravenous administration of calcium^{45}, and phosphorous^{32} resulted in characteristic disappearance curves, with stilbestrol having no significant influence. When the isotopes were administered orally, examination of blood indicated that stilbestrol was without effect in altering the rate of movement of calcium or phosphorus between the gastrointestinal tract, blood, bones and tissues. Following intravenous administration of the radioactivity there was less fecal excretion in the animals receiving stilbestrol, indicating more efficient utilization of absorbed calcium and phosphorus.

In all animals, the urinary excretion of the radioisotopes was negligible. Stilbestrol is considered to affect calcium and phosphorus metabolism either by stimulating bone metabolism directly to induce mineral conservation, by permitting conditions for a more favorable calcium-phosphorus complex in the plasma pool or at the site of mineralization or by a combination of these processes.

FRANK E. RICE

Antibiotic supplementation of animal feeds has been found to improve growth rates in many species. Most observers believe that the effect is one of reducing the intestinal bacterial population thereby decreasing the absorption of toxic metabolic products from this source.

Methionine and Antibiotic Supplementation for Growing Swine at Three Protein Levels. R. F. Sewell and B. C. Keen, Jr. *J. Animal Sc.*, 17: 353, 1958.

Weanling pigs were divided into twelve groups of five pigs each. Rations were composed of yellow corn, solvent soy bean and peanut meals, added salts and vitamin supplements. Feed and water were provided *ad libitum*. Duration of the experiment was seventy-nine days. In those rations containing DL-methionine as supplement, the total calculated content was 2 per cent of the protein. In those containing antibiotic (crystalline chlortetracycline) (Auromycin®), the level

was 10 mg. per pound of feed. The levels of protein used initially were approximately 20, 17 and 14 per cent. These were adjusted downward to 17, 14 and 11 per cent when the pigs averaged 75 pounds and were decreased to 14, 11 and 8.4 per cent when the pigs reached 125 pounds liveweight.

In the absence of supplementation, the 17-14-11 per cent protein combination from weaning to terminal weights produced a higher rate of gain than did a 14-11-8.4 protein combination. But there was no significant difference between the 17-14-11 level and the 20-17-14 feeding level.

There was a highly significant increase in rate of gain due to chlortetracycline supplementation. But there was no difference between the gains at the different protein levels when the antibiotic was added. There was no response to methionine supplementation and there was no significant interaction between the two supplements.

Although methionine is generally recognized to be the limiting factor in high protein oil meals, the authors conclude there is little need for supplementation with this amino acid. FRANK E. RICE

Force feeding of animals has been regarded by research workers as a type of stress which influences utilization of foods. Further evidence supporting this belief is suggested by the differences observed in ad libitum and force-fed rats with respect to body composition.

Changes in Body Composition Attendant on Force Feeding. C. Cohn and D. Joseph. *Am. J. Physiol.*, 196: 965, 1959.

Normal young adult male rats were either force-fed or allowed to eat *ad libitum* a moderate carbohydrate diet for three to four weeks. The force-fed animals were given either the amount of diet consumed by the animals eating *ad libitum* (pair-fed) or 80 per cent of this amount (underfed). After a two-week period of observation, we found that the rats eating *ad libitum* gained 65 gm. of body weight, the pair-fed, force-fed 62 gm. and the underfed, force-fed 40 gm. On the basis of the water, fat and protein content of the skin, viscera and carcass of control animals killed at the beginning of the feeding regimen and of similar constituents of the experimental animals after two weeks of feeding, the composition of the newly formed tissues of the various groups of animals consisted of the following: (1) the rat with free access to food—water = 67.8 per cent, fat = 7.8 per cent and protein = 22.4 per cent; (2) the pair-fed, force-fed animal—water = 55.5 per cent, fat = 23.6 per cent and protein = 17.7 per cent; (3) the underfed, force-fed animal—water = 64.4 per cent, fat = 7.9 per cent and protein = 20.0 per cent. The ratio of calories retained in newly formed tissue to the calories ingested over the two-week period was 11.9 per cent for the animals eating *ad libitum*, 20.6 per cent for the pair-fed, force-fed animals and 9.5 per cent for the underfed, force-fed rats. Force feeding appears to change intermediary metabolic pathways in the direction of increased "efficiency" with resultant greater fat deposition. AUTHORS

Changes in Body Composition During the Course of Acute Anterior Poliomyelitis. A. P. Remenchik, J. M. Dyniecwicz and J. A. Schoenberger. *J. Lab. & Clin. Med.*, 53: 195, 1959.

Antipyrine space, radiosulfate space and total exchangeable potassium were measured simultaneously in forty-four young adults and adolescents with acute anterior poliomyelitis. In some patients repeat determinations were made in seven to ten days. Total body water was found to be increased in males soon after onset of the illness and in females total body water decreased during the illness. Both sexes had the same distribution of water between intra- and extracellular compartments. Intracellular potassium concentrations increased in males early in the illness but did not change in females. G. HOLLIFIELD

HYPER- AND HYPOVITAMINOSIS D

Toxicity of vitamin D may occur when large doses are given to infants or when supplementary treatment is administered to adults. The immediate effect is an increase in serum calcium concentration resulting from increased intestinal absorption of the ion and from increased bone-salt solubility. The clinical findings have been similar to those described in experimental animals.

Hypervitaminosis D in Monkeys: A Clinical and Pathologic Study. S. P. Kent, G. F. Vawter, R. M. Dowben and R. E. Benson. *Am. J. Path.*, 34: 37, 1958.

The experiments to be reported were carried out in about 560 Macaccus mulatta monkeys varying in age from three to nine years and weighing from 2.5 to 10 kg. The animals had previously been used for the study of the effects of radiation and nitrogen mustard. The monkeys were kept on a stock diet supplemented with 162,000 units of vitamin D. Calcium and phosphorus levels were kept high. The experimental diet was discontinued after three months, and the animals were killed at varying intervals thereafter. Clinical symptoms of hypervitaminosis D were weight loss, high incidence of infection of the upper respiratory tract, diarrhea, elevation of blood urea nitrogen and of serum calcium. Complete necropsies and tissue examinations were carried out. Beside residual radiation effects, such as anemia and iron deposits in tissues, the main findings consisted of metastatic calcification of kidneys, salivary glands, lungs, heart and aortas, and, in advanced cases, of gastric mucosa and brain. These findings conform to those of vitamin D intoxication in man and other species. The severity of the changes decreased with increasing interval between discontinuation of the vitamin D supplement and time of death. The

absence of bone lesions was attributed to the high intake of calcium and phosphorus intake.

M. SILBERBERG

An Experimental Histologic Study of Hypervitaminosis D. G. M. Hass, R. E. Trueheart, C. B. Taylor and M. Stumpe. *Am. J. Path.*, 34: 395, 1958.

Male albino rabbits about three months old and weighing approximately 5 pounds were fed Purina rabbit pellets and received either daily, biweekly or triweekly, intramuscular injections of 0.1 ml. radiated ergosterol in peanut oil for six to eight weeks, after which time they were killed. At necropsy systematic tissue examinations were made. Early changes consisted of metastatic calcification; when larger doses had been given, pathologic calcification was preceded by degenerative changes, and large doses produced inflammatory reaction at the same time. These changes were seen particularly in the walls of arteries and periarterial tissue of muscles, heart, respiratory and urinary tract, stomach and tunica muscularis of ileum and colon, and of the endocrine glands, such as the thyroid and thymus. The pituitary, the inner eye and central nervous system did not contain calcium deposits. In the skin the lesions resembled those of fibroelastosis. The bones showed the typical increased resorption. When treatment with vitamin D was discontinued, the inflammatory process subsided and limited repair took place in many degenerated tissues. These signs of improvement were usually preceded by or associated with resumption of osteogenesis.

M. SILBERBERG

The Effect of Cortisone in Experimental Hypervitaminosis D. W. Thomas, Jr. and H. Morgan. *Endocrinology*, 63: 57, 1958.

Since it is known that cortisone is sometimes effective in reducing serum calcium in hypercalcemia an experiment was planned to study the effect of this adrenal hormone in experimental hypervitaminosis D. Groups of rats were given varying doses of vitamin D for seven to fourteen days and histologic examination of bones as well as blood chemical studies were carried out. Large doses of vitamin D led to increased osteoid tissue formation and bone resorption. Other groups of rats were given large doses of cortisone and similar studies were made. Cortisone treatment resulted in increased bone density and reduced cartilage cell proliferation. Finally, cortisone and vitamin D were given simultaneously to rats and it was observed that vitamin D-induced hypercalcemia was not reduced during steroid administration and that the bones showed combined effects of both cortisone and vitamin D treatment. These findings suggest that the interaction of cortisone and vitamin D in the rat may be different than that which occurs in man.

A. B. EISENSTEIN

The relatively high incidence of rickets observed in tropical countries as reported here is of interest considering that solar ultraviolet radiation converts the provitamins to the effective vitamin D. With indoor living, optimum development requires a daily intake of 400 units of the vitamin.

The Prevalence of Rickets at Autopsy in a Subtropical Climate. B. Griffel and S. T. Winter. *J. Trop. Pediat.*, 4: 13, 1958.

In ninety-eight consecutive postmortem examinations of children in Haifa, Israel between two days and two years of age, histologic evidence of rickets was found in sixteen: in ten of these sixteen, prematurity and a prolonged stay in hospital were causal factors. These findings are discussed. The authors stress that particular care in providing adequate vitamin D prophylaxis for premature and hospitalized infants is essential even in the subtropics.

J. C. WATERLOW

Clinical Rickets in the Philippines. E. Stransky and P. O. Dizon-Santos-Ocampo. *J. Trop. Pediat.*, 4: 17, 1958.

The authors describe twenty-two patients with rickets seen at the Philippine General Hospital since 1956, and discuss the physical findings. Rickets is usually associated with general malnutrition: it is generally mild in the tropics and tends to spontaneous cure with increasing exposure to sunshine. The authors suggest that the diagnosis is often missed in the tropics.

J. C. WATERLOW

A Genetic Study of Familial Hypophosphatemia and Vitamin D Resistant Rickets with a Review of the Literature. R. W. Winters, J. B. Graham, T. F. Williams, V. M. McFalls and C. H. Burnett. *Medicine*, 37: 161, 1958.

The main changes of primary vitamin D-resistant rickets as fully described by Fanconi is characterized by a familial, usually dominant genetic pattern, and consists of a chronic absolute vitamin D-resistant hypophosphatemia, elevated serum phosphatase before vitamin D therapy, normo- or hypophosphaturia and marked hypocalcinuria. The disorder manifests itself after the first year of life and is associated with retardation of growth and sturdy body configuration. As a rule serum calcium is normal and alkaline phosphatase elevated; gastrointestinal absorption of calcium and phosphorus is defective and reabsorption of phosphate from the renal tubules diminished. Whether or not the hypophosphatemia is due to hyperparathyroidism secondary to diminished absorption of calcium from the gastrointestinal tract or to an intrinsic renal tubular defect for the transport of phosphate is undecided. Presently, the inheritance pattern of the disorder has been investigated in a large North Carolina kindred, and a primary male-to-male sex-linked dominant pattern was established affecting the levels of serum phosphorus and hypophosphatemia. Asymptomatic female carriers were found in whom the open disease, if present at all, takes a mild course.

M. SILBERBERG

The action of vitamin D appears to involve an alteration in function of alkaline phosphatases in bone, kidney and intestine. Calcification does not occur in the absence of the vitamin despite an abundance of calcium and phosphate. In rachitic animals, normalization of the disturbed areas of bone formation can be observed before calcification occurs upon vitamin D administration.

Early Changes at the Epiphyses of Rachitic Chicks, Following Administration of Vitamin D. L. F. Belanger and B. D. Migicorsky. *J. Exper. Med.*, 107: 821, 1958.

One day old chicks were fed a rachitogenic diet for three weeks. Subsequently the animals were given oral doses of 10,000 or 5,000 units of vitamin D_3; they were killed at intervals varying from two to forty-eight hours after treatment with the vitamin. In a control series chicks were placed on a diet containing 100 units per cent of vitamin D one week prior to sacrifice. The changes occurring in the epiphysis of the tibia were studied by x-ray, microscopically and histochemically, and the uptake of radioactive calcium was determined by autoradiography. Early repair was indicated by resumption of the maturation of the epiphyseal cartilage, while PAS-positive material, presumably chondroitin sulphate, was lost progressively from the matrix. These processes preceded remineralization of the matrix which became demonstrable after two days' treatment when new bony spicules containing PAS-positive material formed. M. SILBERBERG

On the Role of Vitamin D in Calcification. M. Lamm and W. F. Neuman. *Arch. Path.*, 66: 204, 1958.

Rats of the Rochester-Wistar strain were fed a rachitogenic diet high in calcium and low in phosphorus for about four weeks. One group received 7 units/gm. diet calciferol. At the termination of the experiment the long bones were demineralized by immersion in ethylenadianetetraacetic acid at 37°c. for seven days at a pH of 7.4. Thin slides were made and subsequently remineralized by immersion in various calcium x phosphorus products. There were no differences in remineralization (formation of hydroxy apatite) in the bones of normally fed or rachitic animals, respectively. Whether or not the altered calcification of rachitic cartilage and osteoid might be due to an alteration of the crystallization inducing ability of collagen was thus tested. It is concluded that the disturbance of calcification in rickets is something more than mere alteration of the organic matrix. M. SILBERBERG

The Effect of Vitamin D on the Beryllium Rickets of the Rat Incisor. F. M. Wentz, I. Schour and J. P. Weinmann. *Oral Surg.*, 11: 1284, 1958.

The feeding of beryllium carbonate to rats has been shown to cause bone lesions that are similar to rickets. Results in the past have been shown to vary not only with respect to the amount of beryllium fed but also in relation to the initial age and weight of the rats with increasing resistance with increasing age and weight. The present study is a methodically conducted one concerning the influences on the rat incisor and its supporting tissues when beryllium is fed with different levels of vitamin D intake. The high quality of the published work is particularly exemplified by the frequent and consistently excellent photomicrographs. The rats were started on experiment at twenty-four days of age. The first group consisted of twenty rats fed a synthetic diet containing 300 units of vitamin D per 100 gm. of diet which contained 6 per cent beryllium carbonate. Twenty littermates were maintained as pair-fed control animals on the same diet without beryllium carbonate. In the second group, twenty rats were fed the same diet with 6 per cent beryllium carbonate but containing 2,670 units of vitamin D per 100 gm. of diet. Littermate rats were again maintained as pair-fed control animals without beryllium carbonate. Pairs of rats in the two groups were sacrificed progressively from fourteen to 168 days.

Consistently the rats fed beryllium carbonate and the normal level of vitamin D had a severe rachitic syndrome which was characterized by enamel hypoplasia and aplasia, severe retardation in the formation and calcification of dentin, failure of newly formed bone to calcify and resistance of this bone tissue to resorption. These changes were progressive up to the seventieth day after which partial recovery occurred. In the rats fed beryllium carbonate at the same level and a high but not toxic level of vitamin D, the rachitic process was much milder and was confined to rats under three months of age. The following signs were noted: the rate of dentin apposition was slightly retarded and calcification of lingual dentin was impaired with a slightly widened osteoid layer in the alveolar bone.

Among the young rats on the normal levels of vitamin D, the blood phosphorus fell quickly to 2 mg. per cent but increased gradually to 5 mg. per cent in 192 day old rats. However, on the high level of vitamin D intake, blood phosphorus levels were normal. The epiphyseal plate of the tibia was increased in width in beryllium-fed rats on physiologic doses of vitamin D but was normal in rats on the high level of vitamin D. J. H. SHAW